AGENT-BASED MODELING TO ADDRESS EMERGING THREATS FROM ANTIMICROBIAL RESISTANCE TO THE SUSTAINABILITY OF THE BEEF INDUSTRY

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ABSTRACT

Antimicrobial resistance (AMR) is a global public health concern for both human and animal health. The rise in AMR has led to increased scrutiny of antimicrobial use (AMU) in feedlot cattle. With rising concern for prudent AMU in the beef industry, it is essential to understand the potential impact of eliminating parenteral metaphylaxis on AMR and animal health. Further, the impact of transmission of resistant microorganisms and/or the genes encoding for resistance have not been fully investigated in feedlot cattle. In the current thesis, an agent-based model (ABM) was developed under the context of emergence and transmission of AMR within and between feedlot cattle. The objectives of the first experiment were to investigate the influence of on-arrival injectable metaphylaxis options on AMR in feedlot cattle, and of the second experiment were to explore the transmission of resistance determinants through animal-to-animal contact and contact with fecal contamination of the environment. In the first study, resistance prevalence to florfenicol, trimethoprim sulfadoxine (TMS) and tetracycline was assessed in both *M. haemolytica* and *E. coli*. Three different on-arrival metaphylaxis options were considered, including oxytetracycline, tulathromycin or no drugs administered. Resistance prevalence was lower for antimicrobials specifically used to treat BRD following on-arrival metaphylaxis when compared to no injectable metaphylaxis, but only when the antimicrobial used for treatment differ from those used for metaphylaxis. The results suggest there could be less opportunity for resistance selection to antimicrobials used for treating BRD as a consequence of reduced therapeutic AMU due to metaphylaxis administered on arrival. While metaphylaxis will select for resistance to the agent used on arrival, it could potentially minimize selection for resistance to other more medically important drugs used for BRD therapy when compared with no metaphylaxis on arrival. In the second study, the ABM developed in this study successfully replicated previously observed resistance prevalence for *M. haemolytica* and *E. coli* in a typical western Canadian feedlot setting assuming selection due to AMU, transmission of AMR, and a combination of both. The potential importance of transmission of AMR either directly from animal to animal or through the environment must be considered when evaluating expected benefits to antimicrobial stewardship efforts. Further, insights generated from the current ABM can assist policy development and decision making in prevention of AMR.

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LIST OF ABBREVIATIONS

ABM	=	Agent-based model
ADG	=	Average daily gain
AMR	=	Antimicrobial resistance
AMU	=	Antimicrobial use
ARG	=	Antimicrobial resistance gene
BRD	=	Bovine respiratory disease
CCFA	=	Ceftiofur crystalline-free acid
DOF	=	Days on feed
E. coli	=	*Escherichia coli*
ICE	=	Integrative conjugative elements
MDR	=	Multidrug resistance
M. haemolytica	=	*Mannheimia haemolytica*
MIA	=	Medically important antimicrobials
ODD	=	Overview, Design concepts, and Details
ODD + D	=	Overview, Design concepts, and Details + Decision
ODD + 2D	=	Overview, Design concepts, and Details + Decision + Data
TMS	=	Trimethoprim sulfadoxine
WHO	=	World Health Organization

CHAPTER 1
INTRODUCTION AND REVIEW OF LITERATURE

1.1 Antimicrobial resistance

Antimicrobials are any substance of natural or synthetic origin that kill or limit the growth of microorganisms including bacteria, viruses, fungi, protozoans and parasites. During the pre-antibiotic era of the early 1900s, infectious diseases such as pneumonia, typhoid fever, cholera, tuberculosis, and syphilis were rampant. Infections often had high morbidity and mortality (Adedeji, 2016). The discovery of antimicrobials was one of the most significant medical achievements of the 20th century, with the first antibiotic commercialized by British scientist Alexander Fleming in 1920s. In the early 1940s, purified penicillin dramatically improved the prognosis of patients with staphylococcal infection (Rammelkamp and Maxon, 1942). Following the success of penicillin, development and discovery of a wide variety of antimicrobial agents ensued, and appropriately the period between the 1950s and 1970s was named "the golden age of antibiotic discovery" (Aminov, 2010). However, the natural evolution of bacteria combined with widespread use and misuse of antibiotics over time has led to emergence of resistant bacteria.

Antimicrobial resistance (AMR) refers to the ability of microorganisms to withstand the effects of a drug to which they were once sensitive. In other words, a specific drug is no longer able to kill or control the growth of a particular microorganism. Overuse and misuse of antimicrobial medications are among the factors that have contributed to the development of drug-resistant microorganisms (WHO, 2015). Further, the use of antimicrobial agents in feedlot cattle has come under increased scrutiny because of concerns about the potential transfer of resistance genes and bacteria among animals and the environment. Antimicrobial resistance results in infections becoming harder to treat as existing drugs become less effective, ultimately causing increased treatment failure and morbidity. Antimicrobials are useful tools in controlling infectious diseases in feedlot cattle where the spread of infectious disease is more likely to occur (Lhermie et al., 2020). In fact, antimicrobials are widely administered to control many infectious diseases in feedlot (Brault et al., 2019a). It is necessary to examine infectious diseases when discussing AMR as they are the main driver for antimicrobial use (AMU) in the feedlot setting.

1.1.1 Infectious disease in the feedlot

Weaning is a challenging time for beef calves as they are exposed to multiple physical and social stressors. Stressors such as separation from their dams, handling, surgical procedures, transportation, commingling and social reorganization, nutritional changes, and change in housing have all been linked to immunosuppression and increase the risk of subsequent disease such as bovine respiratory disease (Taylor et al., 2010).

Bovine respiratory disease (BRD), also known as "shipping fever", is a major feedlot health problem that accounts for a large proportion of calf mortality and represents an important determinant of AMU (Booker et al., 2008). Bovine respiratory disease is a complex multifactorial disease in calves that is often precipitated by stressful events, and the peak incidence of disease often occurs within the first three weeks after arrival in the feedlots (Wilson et al., 2017). Typically, recently weaned auction-mart-derived calves are considered at high-risk for respiratory disease.

Bovine respiratory disease involves a number of different etiological agents, frequently occurring when a primary viral infection compromises host defenses and allows secondary bacterial pathogens to colonize and replicate within the lower respiratory tract. Bovine respiratory syncytial virus (BRSV), parainfluenza-3 virus (PI3V), and bovine viral diarrhea virus (BVDV) are reported to be inciting viral agents for BRD (Fulton, 2009). These viral etiological agents are often associated with concurrent bacterial infection; *M. haemolytica*, *P. multocida*, *H. somni*, *A. pyogenes*, and *Mycoplasma* spp. are the common bacterial agents in BRD (Callan and Garry, 2002; Cusack et al., 2003). The Canadian Cattlemen's Association (2018) reported that BRD accounts for 65 – 80% of the morbidity and 45 – 75% of the mortality in Canadian feedlots. The wide ranges in morbidity and mortality rates could be attributed to many factors, such as preconditioning, nutrition and immune status of calves. Management practices, including metaphylactic AMU, play a significant role as well.

Morbidity and mortality from BRD in recently weaned calves continue to be the most significant health-related concerns facing feedlot production (Taylor et al., 2010). However, lameness in feedlot cattle has also become another major cause of disease and production losses in recent years (Marti et al., 2016; Davis-Unger et al., 2017, 2019). Lame cattle appear to experience pain and discomfort, as well as having reductions in feed and water intake, diminished body condition score and lower overall health (Desrochers et al., 2001; Terrell et al.,

2014). Increased days on feed (DOF), reduced growth performance, drug expenses, labour to treat lameness, and mortality all contribute to its negative economic and animal welfare costs (Terrell et al. 2014). Jessica Davis-Unger et al. (2017, 2019) studied the prevalence of various types of lameness in Alberta feedlot cattle by examining more than 650,000 health records from 28 feedlots over a period of 10 years. Overall, 14% of cattle were identified as being diseased, and of these diagnoses, lameness accounted for 32% of the cases (overall prevalence of 4.5%). Foot rot was the most common cause of lameness; almost 75% of all lameness diagnoses were attributed to this disease. Joint infections (or arthritis) constituted the second most common lameness diagnosis. However, arthritis was the most common cause of lameness-associated mortality, accounting for almost 50% of lameness-associated deaths (Davis-Unger et al., 2017, 2019).

1.1.2 Antimicrobial use in the feedlot

An understanding of the drivers and reasons for AMU in feedlot cattle is fundamental for a successful reduction of antimicrobial consumption without jeopardizing productivity, animal health and welfare. The introduction of antimicrobials has aided the advancement and productivity of the feedlot industry. Antimicrobials are important tools for maintaining health in feedlot cattle as infections tend to spread rapidly when pens have high stocking densities. Particularly, AMU in feedlot cattle is necessary for the treatment of infections for which alternate management practices (e.g., vaccines) are not available. Therapeutic treatment refers to the use of antimicrobial agents for management of infectious diseases in clinically sick cattle. However, antimicrobial agents are also frequently used for nonspecific means of prevention and treatment of disease (e.g., prophylaxis and metaphylaxis) (Economou and Gousia, 2015).

Prophylaxis and metaphylaxis are used in feedlots for the control of diseases such as BRD. Prophylaxis prevents occurrence of disease or infection by treating animals that are perceived to be at risk of developing disease. Metaphylaxis, however, aims to control the spread of infectious disease to animals in close contact and at considerable risk, such as due to high-stress situations (e.g., after weaning, transport). However, these high-risk animals may already be in the early stage of disease or incubating the disease. Mass antimicrobial metaphylaxis is routine for high-risk cattle at feedlot entry (Brault et al., 2019a). Brault et al. (2019b) reported that 39% of all cattle arriving to the feedlots were categorized as high-risk. Of these high-risk cattle, 95%

received parenterally administered BRD metaphylaxis. In this study, macrolides were the primary antimicrobials administered to high-risk cattle, whereas low-risk cattle were predominately given tetracyclines.

In 2016, approximately 1.0 million kilograms of medically important antimicrobials (MIAs) were sold for animal use in Canada. Of the total mass, 99% was used in food-producing animals and 1% was intended for use in companion animals. Tetracyclines (51%) were the main class of antimicrobials used in livestock, followed by β-lactams (penicillins, 13%), "other antimicrobials" (12%), macrolides (10%), and trimethoprim-sulfonamides (7%) (Government of Canada, 2017). For food-producing animals, antimicrobials are predominantly distributed by weight for use in feed (76% of total usage) as prophylaxis, although the most prevalent classes of antimicrobials vary across the different routes of administration. Unfortunately, data on the usage, proportion and type of antimicrobials sold exclusively for use in cattle is not available. Overall, 78% of the antimicrobials distributed or sold were intended for use in food-producing animals, excluding ionophores and coccidiostats (Government of Canada, 2017).

Brault et al. (2019a) assessed western Canadian feedlots to determine AMU characteristics in the Canadian beef industry. While the treatment and prevention of BRD accounted for most individually administered antimicrobial agents, the authors reported that in-feed AMU was 5-times more common than AMU administered individually in term of the number of animals dosed. Tetracyclines were the most commonly used antimicrobial class, regardless of whether they were administered in feed or individually. Finally, it was concluded that category I, category II, and category III antimicrobial agents contributed to 5%, 39%, and 57% of the individually dosed AMU, respectively. In another study by Brault et al. (2019b), the authors studied AMU in western Canadian feedlots from 2008 to 2012 and reported that macrolides and tetracyclines were the classes of drugs that were most commonly administered parenterally in feedlot cattle.

As production animal antimicrobial sales represented a substantial proportion of overall antimicrobial sales in Canada, the livestock industry is a potential driving force for persistence and spread of AMR (Call et al., 2008). Since antimicrobial sales and distribution data do not accurately indicate how antimicrobials were used, this has led to questions about the role of feedlot contributions to AMR present in the food chain, environment, and human health.

1.1.3 Antimicrobial resistance in feedlots

Microorganisms are arguably the most adaptable organisms on our planet, constantly evolving and efficiently adapting to new environments. Thus, when faced with a barrage of antimicrobial agents, it is not unexpected that a proportion of microorganisms are able to resist their effects. Antimicrobial resistance can evolve naturally via natural selection through random mutation. There was discovery and acknowledgement of resistance even before the widespread usage of penicillin in the late 1940s. In 1940, British biochemists Sirs Ernest Boris Chain and Edward Penley Abraham published a report about an enzyme capable of destroying penicillin, leading to resistance (Abraham and Chain, 1940). However, AMR could also be facilitated by applying environmental pressure on a population. The use of antimicrobial agents provides a powerful selection pressure that acts on a microbial population, selecting for resistance genes that favor the survival of resistant microorganisms.

There is ongoing discussion about the role of AMU in the emergence, spread and persistence of AMR within feedlots. Numerous studies in feedlot cattle have highlighted differences in the associations between resistance and exposure to antimicrobial agents. Some authors have demonstrated an increase in the prevalence of AMR over time spent in a feedlot, while others have not found any associations between AMU and AMR in feedlot cattle. In a study by Inglis et al. (2006), temporal prevalence of AMR in *Campylobacter* species from feedlot cattle was examined. In the four feedlots enrolled in this study, chlortetracycline and oxytetracycline were administered prophylactically in feed to all animals. Most animals also received metaphylactic long-acting oxytetracycline injection upon arrival. The authors reported that resistance to tetracycline and doxycycline in *Campylobacter* species was less than 11% on arrival, but rapidly increased (up to 10-fold) over the course of the feeding period. Moreover, the proportion of cattle with tetracycline resistant isolates of *Campylobacter* (< 2% on arrival) also increased drastically (7 – 42%). Similar to these findings, Englen et al. (2005) reported 52% overall resistance to tetracycline in *Campylobacter* species from feedlot cattle. Specifically, the *C. jejuni* isolates (49%) were found to be less resistant to tetracycline than the *C. coli* strains (66%). However, this study did not monitor antimicrobial exposure in these cattle and thus impact of antimicrobial administration on AMR was not examined.

A randomized control field trial in western Canada by Checkley et al. (2010) examined the influence of in-feed and subcutaneous administration of oxytetracycline on resistance in

commensal fecal *E. coli* isolated from feedlot cattle. Two hundred and eighty-eight newly weaned, auction mart derived steers were randomly assigned to one of three treatments. Group 1 received no antimicrobials on arrival. Group 2 was fed oxytetracycline in the starter ration for 14 days, whereas group 3 was administered oxytetracycline subcutaneously on arrival. Fresh fecal samples were collected from each steer on days 0, 7, 15, 35, 70, 100, 150, and preslaughter. Three generic *E. coli* isolates per animal were randomly chosen and evaluated for susceptibility to seven antimicrobials. The authors reported that in-feed administration of oxytetracycline was associated with higher levels of resistant *E. coli* in feces compared to subcutaneous treatment at day 15. Overall, animals having at least one isolate resistant to tetracycline, sulfamethoxazole, and ampicillin prior to slaughter were 6.4, 4.2, and 3.8 times higher, respectively, following in feed administration, compared to when oxytetracycline was used in feedlot cattle parenterally on arrival.

Other researchers have suggested that AMU has transient and relatively short-term impacts on the prevalence of resistant isolates in fecal *E. coli*. Parenteral administration of ceftiofur crystalline-free acid (CCFA) to feedlot steers was associated with a temporary increase in the recovery of multiple-resistant variants of *E. coli*, with a return to the pre-treatment levels approximately 2 weeks after completion of CCFA administration (Lowrance et al., 2007). The temporality of resistance elevation was also reported for chlortetracycline treatment in feedlot cattle by Platt et al. (2008). Chlortetracycline in rations fed to feedlot cattle was associated with a transiently increased proportion of resistant fecal *E. coli* and *Enterococcus* isolates. Chlortetracycline in feed was also associated with a decreased likelihood of recovering ceftiofur-resistant *E. coli*. The authors hypothesized that exposure to tetracycline may have provided a competitive disadvantage for ceftiofur-resistant bacteria in that specific population. However, Checkley et al. (2008) found no associations between AMU and AMR in feedlot cattle receiving antimicrobial metaphylaxis on arrival without in-feed prophylaxis.

A number of observation studies have reported AMR prevalence in BRD-related bacteria such as *M. haemolytica*, *P. multocida*, and *H. somni* (Klima et al., 2014a,b; Timsit et al., 2017). Timsit et al. (2017) conducted a study in mixed breed beef steers and heifers that were at high-risk of developing BRD in western Canada. All cattle received metaphylactic injection of tulathromycin on arrival and prophylactic in-feed treatment of chlortetracycline within the first 21 DOF. Cattle with more than one visual BRD sign (e.g., lethargy, nasal or ocular discharge,

cough, tachypnea or dyspnea), a rectal temperature ≥ 40°C and abnormal lung sounds were defined as BRD cases and pen-matched with control calves with no visual BRD signs, rectal temperature < 40°C and no abnormal lung sounds at auscultation performed by a veterinarian. Upon enrollment in the study, cattle were sampled by transtracheal aspiration (TTA). The authors concluded that *M. haemolytica* and *P. multocida* isolated from feedlot cattle showed high levels of resistance (> 70%) against tulathromycin and oxytetracycline, with the majority of resistance found in cattle with BRD.

Klima et al. (2014a) conducted a similar study where *M. haemolytica* was isolated from the nasopharynx of feedlot cattle upon entry into and exit from the feedlot. Tetracycline resistance (18%) was the most common resistant phenotype, followed by neomycin (15%). All isolates were found to be susceptible to ceftiofur, enrofloxacin, and danofloxacin. However, it was observed that *M. haemolytica* strains isolated from cattle with clinical respiratory disease was more likely to be resistant to at least one antimicrobial compared to healthy cattle (37% vs. 2%). Further, *M. haemolytica* isolates from diseased cattle were disproportionately represented by serotypes 1 (71%) and 6 (20%), whereas healthy cattle were mostly characterized by serotype 2 (76%). The authors suggested that the dominant serotype linked to BRD may have had a competitive advantage due to AMR.

Another study by Klima et al. (2014b) investigated integrative conjugative elements (ICE) mediated AMR in BRD-related bacteria. Isolates of *M. haemolytica*, *P. multocida*, and *H. somni* from BRD mortalities were collected. The results suggested high overall rate of resistance with 72% of *M. haemolytica* and 50% of *P. multocida* isolates exhibiting AMR, and 45% of all bacterial isolates displaying resistance to three or more antimicrobials. Of these resistant isolates, 30% and 13% were resistant to more than seven antimicrobial classes for *M. haemolytica* and *P. multocida*, respectively. Furthermore, the authors identified the presence of ICE carrying AMR encoding genes with up to seven antimicrobial classes in these BRD pathogens.

Holman et al. (2018) reported a positive association between AMU and AMR in feedlot cattle. In this study, injection on arrival was compared against no metaphylactic treatment; all animals received in-feed chlortetracycline and monensin sodium. The authors reported that resistance gene *tet(H)* in the nasopharyngeal microbiome significantly increased following oxytetracycline injection at feedlot entry. These resistance genes can then be transferred via plasmid exchange between animals (Harbottle et al. 2006).

Other studies completed in feedlot cattle have shown resistance to be an uncommon occurrence. Alexander et al. (2013) evaluated *M. haemolytica* isolated from feedlot cattle with a known history of AMU over a three-year period. The authors found that tulathromycin resistance was exceptionally low (0.4%) four years after the approval of tulathromycin for treatment and prevention of BRD in Canada. Similarly, Noyes et al. (2015) found the prevalence of AMR in BRD-associated bacteria to be relatively low in feedlot cattle, even when antimicrobial agents are used. The authors also found no association between antimicrobial exposure among sampled cattle or their penmates, and resistance to any single drugs. However, antimicrobial exposure in individual animals was shown to increase the risk of isolating both susceptible and multiple-resistant *M. haemolytica* from penmates.

Vikram et al. (2017) studied AMR in feedlot cattle post-slaughter. In total, 36 lots of feeder cattle conventionally raised (CONV) and "raised without antibiotics" (RWA) were sampled from a commercial beef processing plant, respectively. Fecal samples retrieved from the colon of each animal were collected following evisceration and subsequently tested for presence of resistant bacteria. While AMR levels were similar between treatment groups, cephalosporin-resistant *E. coli* and erythromycin-resistant *Enterococcus* sp. concentrations were significantly higher in the CONV group compared to RWA. However, there were no significant differences between CONV and RWA systems in relation to macrolide or tetracycline resistance, and the authors concluded that reductions in AMU were not likely to reduce the levels of AMR for these two drug classes.

Additionally, Vikram et al. (2018) also collected ground beef samples from three food service supply facilities to evaluate the effect of CONV and RWA production systems on AMR levels. A total of 191 and 179 samples were collected from ground beef produced by CONV and RWA systems, respectively. Tetracycline-resistant *E.coli* concentrations were significantly higher in CONV systems, although the effect of production system differed by supplier. However, AMR genes tet(A) and tet(B) were significantly higher in the samples derived from RWA ground beef. The authors reported that sample microbiomes differed more by supplier versus the production system, and therefore concluded that AMU has a minimal impact of AMR found in ground beef products.

1.1.4 Challenges in monitoring epidemiology of AMR in feedlots

A better understanding of the epidemiology of AMR emergence and spread in feedlots will provide an essential foundation for successful mitigation strategies. However, research that critically evaluates the potential relation of resistance to AMU or AMR emergence in feedlots is limited. Both randomized controlled clinical trials and observational studies are commonly used for epidemiological studies. Unfortunately, many of the randomized controlled clinical trials in feedlots have focused on the efficacy of different antimicrobial agents as treatment for respiratory disease or evaluating the effects of metaphylactic treatment on morbidity and mortality. Moreover, previous studies which emphasized AMR in feedlots are primarily longitudinal observations, and are generally restricted by a number of limitations.

There are numerous challenges to studying relationships between AMU and AMR. First, confounding factors can be particularly problematic in observational studies. Unmeasured confounding factors such as feedlot management practices, previous exposure to antimicrobial agents, route of exposure and environment could affect how well AMU predicts AMR. Further, clustering of data is common in observational studies involving livestock species due to the way in which animals are housed and managed. Clustered data can also arise when data are compiled across multiple studies, with each study providing a distinct cluster of data. Consequently, individual animals (or groups of animals) should no longer be considered independent from one another, and such dependency must be accounted for in the study design or adjusted for in the statistical analysis (Sargeant and O'Connor, 2014; O'Connor et al., 2016a). Thus, observational studies have limited value in assessing potential causal relationships. By contrast, randomized controlled studies are considered the "gold standard" of epidemiologic studies and provide the most reliable evidence of causation if properly designed. A well-designed randomized controlled trial can effectively minimize bias and confounding factors (Spieth et al., 2016). However, randomized controlled studies are expensive, and resources (e.g., labour, time, animals) are often limited in research settings. Further, a common limitation with randomized controlled studies is narrow treatment protocols that don't reflect commercial industry practices. Additionally, research studies conducted on commercial cattle feeding operations are often challenged by limited labour resources and increased time required to process cattle for sample collection.

A major barrier to understanding AMU and resistance in feedlot cattle is the difficulty in obtaining complete and accurate AMU records. In order to identify the specific underlying drivers for the development of resistance, accurate and detailed data on AMU is of critical

importance. Comprehensive AMU data is needed to ensure that true exposure-resistance associations are not overlooked. However, AMU data collection with sufficient detail can be logistically challenging for a number of reasons (Landers et al, 2012). In contrast to swine and poultry operations, the time that cattle are intensively managed in a feedlot is relatively long and can vary significantly. Factors such as weight at placement, growing conditions, and desired finished condition can change the length of time cattle spend in a feedlot. Further, animals are typically re-sorted at some frequency for the purpose of marketing homogenous groups of cattle. Therefore, following individual animals and specific pen groups can be challenging due to re-sorting. Finally, AMU has been measured using a large diversity of metrics due to lack of measurement standardization. This has created variation in the interpretation of AMU, posing considerable difficulty to the comparability of data across studies (Benedict et al., 2012; Brault et al., 2019b). Therefore, it is difficult and sometimes impossible to evaluate the association between AMU and AMR with accuracy.

Large-scale epidemiological studies with coordinated effort by all stakeholders are necessary to understand and manage the emergence and spread of AMR in feedlot cattle. All relevant information, including the use of antimicrobial agents, modes of administration and resistance prevalence in all potential bacterial reservoirs should be considered. Previous experimental designs for examining the epidemiology of AMR in feedlots are often cost prohibitive and unable to fully assess exposure-resistance associations. Fortunately, new technology has become available that enables researchers to simulate real world conditions using computational dynamic models as a valuable supplement to traditional approaches.

1.2 Dynamic computational models

A dynamic models can be defined as a simplified representation of a real-world entity that evolves over time, where the evolution at any given time is dictated by the state of the system at that time. These models can account for time-dependent changes in the state of the system and can contain internal dynamic variables whose values change over time. Furthermore, transition rules and/or external parameters are typically defined to govern the behaviour of the model (Abar et al., 2017). Dynamic modelling can be carried out using computational modelling approaches, which refer to the use of computer program to describe and predict the dynamic relationships between interacting components of a complex system. Dynamic computation

models rely on the power of computers to explore dynamics out of the reach of pure mathematical methods by specifying the potential transition rules using formal logics. The goal of dynamic models is to explain important dynamic phenomena and processes, and they are commonly used as a theory-building tool when dynamics of interest are too complex and/or relatively few empirical data exist (Davis et al., 2007).

There are several dynamic modelling approaches for investigating complex real-life systems characterized by dynamic nonlinear relationships. System Dynamics and agent-based modelling are the two most commonly used methodologies in modelling a complex dynamic system. System Dynamics models are often used to build higher-level theories that explain the dynamic changes in systems. They consist of stock-flow structures linked by positive or negative causal links into positive and negative feedback loops (Ip et al. 2013). System Dynamics modelling focuses on the relationships between the individual components rather than focusing on the separate components in isolation. This type of dynamic models is particularly well suited to modelling high-level system behaviour in large populations. They seek to explain system behaviours by understanding the internal structure of a system rather than focusing on factors external to the system (Richardson, 2011). System Dynamics models typically consists of a system of differential equations that express relationships among stock and flow. In agent-based modelling, a system is modeled as a collection of autonomous decision-making entities called agents. Each agent individually assesses its situation and makes decisions on the basis of a set of properties and decision rules (Gilbert and Troitzsch, 2005; Auchincloss and Diez Roux, 2008). Through simulating the agent-based model (ABM), one can observe emergent phenomena resulting from interactions with other agents and from other components of the environment. That is, it allows the emergence of population-level phenomena that can be different from what would be expected based only on the aggregation of individual behaviours (Bonabeau, 2002).

Computational dynamic models are well suited to investigate the dynamics of AMR because the approach takes into account the complexity, dynamic nature and system-wide behaviour associated with AMR. These models have the ability to verify hypotheses using theoretical scenarios, or to generate estimates when available data is limited. By running simulations using a dynamic model, researchers are able to explore the effectiveness of potential interventions without the expense or possible adverse consequences of implementing new interventions in the real world. Furthermore, the simple act of creating a model elicits clear

statements related to the area of focus, leading to valuable discussions and a better understanding of the research question.

1.2.1 History of use for dynamic simulation modeling

Dynamic modelling is a powerful simulation modelling technique that has seen a number of applications and continues to find new applications in many fields. An explosive growth in computational and information processing has enabled the emergence of more diverse research in the field of modelling and simulation. Both System Dynamics and agent-based modelling have been applied to many domains, including ecology (Planque et al., 2014; Martin and Schlüter, 2015), economics (Marzouk and Azab, 2014; Lee et al., 2019), social sciences (Epstein, 2002; Bruch and Mare, 2006; Magliocca et al., 2013), and supply chains (Ge et al., 2004; Feng, 2012).

Over the past several decades, dynamic simulation modeling has become an essential tool in analyzing the epidemiological characteristics of infectious diseases in both humans and animals (Keeling and Rohani, 2008). As early as 1927, Kermack and McKendrick developed an epidemic model consisting of three ordinary differential equations (ODEs) governing the movements between susceptible, infected and recovered individuals in a given population. This classic model is considered the basis from which many other compartmental models were developed. Thenceforth, many dynamic models in infectious disease epidemiology have been developed. In human health, these include the control and prevention of measles transmission (Enanoria et al., 2016), strategies to prevent an H5N1 influenza (Ferguson et al., 2005), vaccination strategies against an influenza pandemic (Lee et al., 2010), and impact of interventions to reduce human immunodeficiency virus (HIV) incidence (Kok et al., 2015; Escudero et al., 2016). For animal-related diseases, studies that examine social risk factors associated with feline immunodeficiency virus (FIV) transmission (Fouchet et al., 2009), effect of dog demography on rabies vaccination (Chidumayo, 2018), beef cattle production and transportation (Yang et al., 2019), and between-farm transmission of the porcine reproductive and respiratory syndrome (PRRS) virus (Thakur et al., 2015) are some of the examples.

Beyond the transmission of infectious diseases, computational dynamic models are well suited to investigate the dynamics of other important public health challenges. AMR is complex, dynamic in nature and involves system-wide behaviour, making computational dynamic models valuable tools for this type of research.

1.2.2 Types of dynamic simulation models used for simulating AMR in livestock

The vast majority of studies examining AMR using dynamic simulation models involve humans (Temime et al., 2012; Almagor et al., 2018). Only a handful of scientific studies have been conducted using dynamic simulation models to investigate the impact of AMR in livestock. These studies have been conducted primarily using deterministic models based on sets of ODEs. An ODE is an equation which involves derivatives of one or more dependent variables with respect to a single independent variable.

A few researchers have undertaken the challenging task of developing dynamic models to study the impact of AMU on emergence and/or transmission of AMR within pig production units. Abatih et al. (2009) proposed a deterministic model in which pigs were subdivided into three groups: uninfected, infected with predominantly drug-sensitive bacteria, or infected with predominantly drug-resistant bacteria. The authors concluded that the proportion of finisher pigs with resistant bacteria before transport to slaughter could be decreased through a reduction of the transmission rate or increasing the spontaneous clear-out rate for resistant bacteria.

The effect of tetracycline treatment on the evolution of the *E. coli* microbiota in pigs was modelled by Græsbøll et al. (2014). Antimicrobial resistant *E. coli* were reported to persist in a pig population through competitive growth between strains in individual pigs, as well as through transmission between pigs within a pen. Moreover, resistant bacteria could quickly become dominant when antimicrobial treatment was initiated. However, resistant strains were found to have a fitness cost of more than 10% reduction in their growth rate.

In a similar deterministic modelling study, the effect of intramuscular ampicillin on levels of resistance in commensal *E. coli* in the intestine of nursery pigs was evaluated (Ahmad et al., 2016). This study determined that resistance levels were influenced by the number of competing strains and short treatment duration resulted in fewer resistant *E. coli*. Furthermore, resistant *E. coli* strains were excreted at a higher rate, resulting in faster return to pre-treatment equilibrium. In contrast to results reported by Græsbøll et al. (2014), this study suggested that ampicillin resistant *E. coli* did not carry a fitness cost for their resistance.

Schulz et al. (2018) developed an agent-based Monte Carlo simulation model to assess the impact of pig movement on the spread of livestock-associated methicillin-resistant *Staphylococcus aureus* (LA-MRSA) among pig herds in Denmark. The model suggested animal

movements alone could not account for the observed increase in LA-MRSA positive herds in Denmark. However, the model was able to mimic the development of LA-MRSA positive pig herds similar to the trend observed in Denmark by combining animal movements and indirect contact (i.e., among herds, via humans or equipment).

Similarly, deterministic models of different complexities have been developed to understand the dynamics of plasmid-mediated AMR in enteric commensals of beef cattle. Volkova and others (2012) developed a deterministic model to examine the dynamics of ceftiofur-sensitive and resistant commensal *E. coli* in the large intestine of cattle. The authors concluded that ceftiofur resistance can persist in enteric *E. coli* between ceftiofur therapies, continuing by vertical and horizontal transfer of plasmids carrying resistance genes. During parenteral ceftiofur treatment, resistant enteric *E. coli* increased in overall number whilst the number of sensitive enteric *E. coli* decreased. The bacterial subpopulations were affected by fitness cost of plasmid carriage by resistant bacteria, inflow and outflow of *E. coli* from the large intestine. Particularly, Volkova et al. (2012) determined that the rate of horizontal transfer of plasmids between enteric *E. coli* and the replacement rate of resistant *E. coli* acquired through ingestion are important determinants for maintenance of resistance in the absence of ceftiofur pressure. Volkova et al. (2013) conducted a follow-up study to address approaches for controlling plasmid-mediated AMR in enteric commensal *E. coli* of cattle. The authors suggested that interventions capable of inducing plasmid loss in resistant enteric *E. coli* or reducing the maximum number of *E. coli* in the large intestine were both efficient in reducing the fraction of resistant enteric *E. coli* at slaughter age.

Most existing dynamic models examining AMR in livestock are based on sets of ODEs. These models have limitations, including the lack of stochasticity, the inability to represent individual behaviours and previous experiences, and difficulty representing emergent properties, which are all common characteristics of AMR. Additionally, the analysis of ODE models is conducted at a high level of aggregation of the system entities. Agent-based modelling is an alternative paradigm to ODE modelling that overcomes these limitations.

1.3 Agent-based modeling in AMR research

While previous research has been conducted to evaluate the relationship between AMU and AMR in livestock, there are still substantial limitations in our understanding of the

emergence and transmission of AMR. Antimicrobial resistance in populations involves an intricate interplay between hosts, bacteria, and environmental factors (Woolhouse et al., 2015). Further, the individual-level effects can compound at the population level because of transmission. An alternative comprehensive approach is needed to improve our understanding of the selection and transmission of AMR, enabling better management practices, improved detection and enhanced prevention strategies.

Agent-based modelling is a powerful modelling technique appropriate to study complex adaptive systems. An ABM simulates behaviours and interactions between autonomous entities referred to as agents. Furthermore, stochasticity influences behaviours and changes in the model, allowing it to unfold in a probabilistic manner. Changes in agent and environmental characteristics may also be altered over time through feedback and adaptation, which are features that traditional statistical models cannot accommodate (Bonabeau, 2002; Rutter et al., 2017). By modeling the relationships on the level of individuals in a rule-based way, agent-based modelling allows the user to produce characteristic features of the system as emergent phenomena without having to make assumptions regarding the aggregate system properties. Thus, agent-based modelling might be more suited than aggregate System Dynamics models for the simulation and study of some questions about AMR.

Agent-based models are increasingly used to guide public health interventions for the prevention of AMR development and transmission. However, the use of ABMs as they pertain to AMR in agriculture and food system settings is still very limited (Ramsay et al., 2018). Most of these models have been used to explore the spread of multi-drug resistant bacteria in community or hospital settings (Sébille and Valleron, 1997; Kardaś-Słoma et al., 2011; Suthar et al., 2014; Almagor et al., 2018) or the effect of intervention strategies (D'Agata et al., 2007; Kardaś-Słoma et al., 2013). Given the untapped potential of agent-based modelling, it seems likely that ABM could soon yield substantial contributions to AMR research in livestock production as a supplement or an alternate experimental approach.

1.3.1 Benefits of agent-based modeling

Agent-based models offer endless possibilities to explore complex heterogeneous problems and provide valuable information about the dynamics of the depicted real system. These models provide a platform to examine the interactions between individuals and their

interactions with the local environment; these interactions often result in the emergence of unanticipated behaviours. Bonabeau (2002) concluded there are three main benefits of ABM over other modelling techniques. First, ABMs do not rely on equations to govern system dynamics, but rather they consist of dynamically interacting rule-based agents. This allows for the conceptualization of a system as it would exist in the real-world. Further, this form of dynamic modelling is flexible. Agent-based models can be refined relatively easily as new information becomes available. Most importantly, the agent-based modelling approach is capable of capturing emergent phenomena.

Compared to other modelling approaches, ABMs are a more natural way for describing and simulating a system composed of real-world entities (Bonabeau, 2002). They often make the model seem more akin to reality. For example, it is easier to conceptualize and model individual calf interactions in a feedlot pen than it is to produce equations that govern the dynamics of calves' movement. Further, ABMs have the ability to extract abstract information from the dynamics generated by the simulation and to visualize them in real-time. Thus they can be used to visually convey the behavior of the model clearly and quickly to stakeholders. Moreover, individual behaviour is complex. Although animal behaviour can often be defined through complex differential equations, the differential equations that are required become more complex as the complexity of individual behaviour increases. Lastly, stochasticity is applied strategically in ABMs to govern agents' behaviour, whereas an arbitrary factor is commonly added to differential equation in other types of dynamic models.

The flexibility of ABM is a major strength of this modelling approach. By explicitly modeling each real-world entity as a separate "agent", ABMs allow enormous flexibility in capturing heterogeneity across agents (Bonabeau, 2002). Agents themselves may be modelled on different levels of scale (e.g., "animal" agents vs. "feedlot" agents). Furthermore, ABMs allow for complex depiction of mechanisms within an agent (e.g., selection of resistance) that could be influenced by external factors (e.g., drug use) or other agents (e.g., animal to animal or environment to animal transmission). Additionally, agent-based modelling techniques are particularly useful at modelling interaction and adaptation. By modelling populations of individuals, ABM can capture the interaction of agents with each other and with their coevolving environments. Finally, when new empirical data become available, ABM can be easily refined to

reflect current knowledge, including through the incorporation of aspects of heterogeneity that are difficult to incorporate in aggregate modeling.

The greatest benefit offered by ABMs compared to other computational approaches are their ability to capture emergent properties of the system (Bonabeau, 2002). Agent-based models do not require knowledge of the aggregate behaviours. Instead, rules and constraints are used to describe predictable behaviour at the individual level, and the interactions among agents and their environment often aggregate to create unexpected emergent phenomena through feedback loops and adaptation. As suggested by Bonabeau (2002), "the whole is greater than the sum of its parts" because of the interacting agents. In other words, ABMs can provide both individual and aggregate level detail at the same time. Further, real-world complex problems are often characterized by substantial heterogeneity among individuals. Agent-based models can simulate a heterogeneous population, whereas equational models typically make assumptions of homogeneity and tend to smooth out fluctuations.

1.4 Summary

Antimicrobial resistance has emerged as an urgent health threat to both humans and animals. The need for action to avert this global crisis is imperative. There is general scientific consensus that AMU in a variety of settings contributes to the burden of AMR. However, an exact quantification of the public health burden attributable to AMU in livestock production compared to other sources remains challenging. Few issues are as complex and multifactorial as the issue of AMU on AMR in food-producing animals, which has become the subject of increased attention over the past decade. In particular, the link between AMU and AMR in feedlot cattle remains contested by critics, as a limited number of studies are available. Given divergent stakeholder interests and inadequate research in feedlot cattle to date, additional research is needed. However, large-scale randomized controlled experiments are difficult to conduct in a commercial setting due to limited resources. Therefore, alternative methods for evaluating the relationship between AMU and AMR in feedlot cattle are needed.

Dynamic computational modelling provides a mean to untangle the real-world complexity to uncover the general principles and mechanisms of AMR. Specifically, ABMs can be a complement to classical empirical research methods. These models provide a platform for *in silico* experiments that help researchers generate refined hypotheses and interpret empirical

observations. Models also make empirical experimentation more efficient, and *in silico* experiments can be conducted at a reduced cost while avoiding ethical dilemmas. Furthermore, it is possible to systematically generate different scenarios and conduct experiments in a simulation environment.

Together, use of traditional empirical research and ABMs represent an iterative process that combines theory development and testing, and this is necessary to reach a deeper understanding about complex dynamic issues such as AMR. There is no doubt that public concerns around AMR in food animals are growing and demand for antibiotic-free meat is increasing among consumers. An alternative comprehensive approach is needed to improve our understanding of the selection and spread of AMR in feedlot cattle; ABMs are well suited to study these AMR dynamics.

1.5 Research Hypothesis and Objectives

It was hypothesized that restriction/elimination of AMU for disease prevention from beef industry would have a negative impact on the sustainability of beef industry. In other words, the incidence of AMR may decrease in the absence of AMU for disease prevention but a corresponding reduction to animal performance and increase in AMU for disease treatment may also be observed if effective alternative disease management strategies are not identified. In addition, it was hypothesized that environmental contamination and animal-to-animal contact potentiates the spread of AMR.

The objective of Chapter 2 was to explore the potential impact of injectable metaphylactic use on the prevalence of AMR in feedlot cattle in a typical Canadian feedlot using an ABM. While antimicrobials used for metaphylaxis may select for antimicrobial resistant pathogens, the corresponding reduction in BRD incidence could result in lower use of more important classes of antimicrobials used for BRD therapy. However, Chapter 2 only considered the role of AMU on AMR prevalence, when in fact the influence of contagious AMR spread is also an important transmission consideration in feedlot cattle. Accordingly, the objective of Chapter 3 was to assess the importance of contagious spread of AMR through animal-to-animal contact and environmental contamination in explaining observed trends in AMR prevalence as compared to selection due to AMU alone. *M. haemolytica* was considered as a surrogate for respiratory pathogens and *E. coli* as a surrogate for fecal commensal organisms.

Different from traditional modelling approaches, ABMs can represent entities of different types, their heterogeneity, actions, and interactions. In summary, it is anticipated that results obtained from this study will improve our understanding of AMR in feedlot cattle. Agent-based modelling has the potential to integrate components of AMR surveillance and research into more comprehensive, cohesive, meaningful information that is needed by policy makers to understand AMR and the impact of interventions.

CHAPTER 2
AN AGENT-BASED MODEL EXAMINING THE ASSOCIATION BETWEEN METAPHYLACTIC INTERVENTION AND ANTIMICROBIAL RESISTANCE IN A WESTERN CANADIAN FEEDLOT

Michelle Thompson, Nathan Erickson, Sheryl Gow, Nathaniel Osgood, Cheryl Waldner

This chapter investigates the effect of antimicrobial agents administered as BRD metaphylaxis on AMR in feedlot cattle. Little work has been done to examine the potential impact of eliminating parenteral metaphylaxis in cattle arriving at the feedlot. In recent years, preventative use of antimicrobials has come under intense public scrutiny because of concerns that mass medication could promote the emergence of resistant organisms. Further, the World Health Organization (WHO) has recommended that critically important antimicrobials should not be used for disease prevention without individual animal diagnosis in an effort to reduce the use of critically important antimicrobial agents in food producing animals. The key finding of this chapter was that metaphylactic tulathromycin or oxytetracycline treatments reduced resistance prevalence to antimicrobials used to therapeutically treat BRD. If on-arrival metaphylactic AMU in high-risk calves was eliminated without suitable health management alternatives, it could result in greater disease incidence and subsequently higher use of MIAs for therapeutic treatment. Therefore, we suggest that metaphylaxis is warranted to reduce disease incidence throughout the feeding period. However, products that have lower importance to human medicine should be selected for metaphylaxis where feasible to minimize the potential public health impact since an increase in resistance prevalence was observed within the same drug class as the antimicrobial administered for metaphylaxis. Thus, resistance prevalence estimates emerging from Chapter 2 are based on selection from AMU alone. Chapter 3 focuses on bidirectional exchange of antimicrobial resistant microorganisms occurring indirectly via the immediate environment and directly through contact with other animals in close proximity.

Author contributions: Michelle Thompson was responsible for model design and implementation, data analysis, and manuscript preparation. Waldner, Erickson, Gow, and Osgood were responsible for study design and manuscript review.

2.1 Abstract

Metaphylaxis is a common feedlot cattle health management practice that has been shown to substantially reduce morbidity and mortality attributed to bovine respiratory disease (BRD) (Brault et al., 2019a). However, antimicrobial use (AMU) provides selective pressure for emergence and propagation of resistant organisms (Aarestrup, 2015). With rising concern for prudent AMU in the beef industry (Tang et al., 2017; WHO, 2017; Hannon et al., 2020), it is essential to understand the potential impact of eliminating the use of on-arrival parenteral metaphylaxis with regards to antimicrobial resistance (AMR) as well as animal health. The objective of this study was to explore how different options for on-arrival injectable metaphylaxis might influence AMR. This study was conducted using a stochastic agent-based dynamic simulation model to characterize the evolution of the prevalence of AMR in *Escherichia coli* (*E. coli*) and *Mannheimia haemolytica* (*M. haemolytica*) in feedlot calves receiving parenteral tulathromycin or oxytetracycline compared with calves receiving no metaphylaxis. The prevalence of *E. coli* resistant to florfenicol and trimethoprim sulfadoxine (TMS) during the feeding period was lowest when tulathromycin was administered on-arrival, and highest when no injectable metaphylaxis was used. In contrast, *E. coli* resistance to tetracycline was more common when oxytetracycline was used on-arrival than with either tulathromycin use or no metaphylaxis ($P < 0.05$). Similarly, *M. haemolytica* florfenicol resistance was lowest following tulathromycin use and differed significantly between metaphylaxis options for days 20 and 90 ($P < 0.05$), although no differences between treatments were observed on day 225. The lowest TMS resistance prevalence for *M. haemolytica* was also observed with tulathromycin use, followed by oxytetracycline use, while no metaphylaxis resulted in the most resistance ($P < 0.05$). Finally, the prevalence of tetracycline resistant *M. haemolytica* was significantly lower throughout the feeding period following tulathromycin use and no metaphylaxis compared to oxytetracycline ($P < 0.05$). Resistance prevalence was lower for drugs specifically used to treat BRD following on-arrival metaphylaxis when compared to no injectable metaphylaxis, but only when the products used for treatment differ from those used for metaphylaxis. This model suggests there could be less opportunity for resistance selection to products used for treating BRD as a consequence of reduced AMU for therapeutic purposes due to the metaphylaxis on arrival. Metaphylactic AMU remains the most effective option for controlling BRD in feedlot cattle. While metaphylaxis will select for resistance to the agent used

on arrival, it could potentially minimize selection for resistance to other drugs used for BRD therapy. This result assumes the prevalence of integrative conjugative elements (ICE) or plasmids facilitating multidrug resistance (MDR) is relatively low in the population and that selection due to AMU is a more important driver of resistance emergence in feedlot cattle than infectious transmission of resistant organisms.

2.2 Introduction

The success of a feedlot is greatly dependent upon the health of the cattle (Irsik et al., 2006). The use of antimicrobials has been instrumental in controlling and preventing diseases in feedlot production (Brault et al., 2019a). Antimicrobials are used in western Canadian feedlot operations for three main purposes: 1) therapeutic use for treatment of infections in clinically sick calves, 2) metaphylaxis for treatment of mixed groups of infectious calves with clinical symptoms and exposed calves potentially incubating the disease, and 3) prophylactic use in apparently healthy calves to prevent the occurrence of disease or infection based on history and clinical judgement (McEwen and Fedorka-Cray, 2002; Cameron and McAllister, 2016). In recent years, prophylactic and metaphylactic use of antimicrobials has come under intense scrutiny because of concerns that mass medication could promote the emergence of resistant organisms (Tang et al., 2017; WHO, 2017; Hannon et al., 2020). In response to the issue of AMR, the WHO has recommended that healthy animals only be administered antibiotics if disease has been diagnosed in other animals within their herd to reduce the use of medically important antimicrobials (MIA) in food producing animals (WHO, 2017). They have also identified a list of antimicrobials commonly used in North American feedlots that should only be considered for use if diagnostic testing has demonstrated that there is no other effective option.

Bovine respiratory disease is the most common and extensively studied disease of feedlot cattle (Ives and Richeson, 2015; Brault et al., 2019a). It can lead to significant economic losses for feedlot producers through mortality, morbidity, decreased performance and reduced carcass values (Ives and Richeson, 2015; Brault et al., 2019a). The transition period between less intensive production settings (e.g., pasture or backgrounding) to feedlot housing is one of the most critical times in terms of beef cattle health and nutritional management (Taylor et al., 2010). During this transition, animals are exposed to stressors that can include weaning, long distance transportation, processing, dietary changes, and exposure to infectious agents from mixing or commingling with animals from different sources (Sanderson et al., 2008; Checkley et al., 2010; Taylor et al., 2010; Smith et al., 2020). Alone, these stressors may be insufficient to trigger BRD, but together they predispose animals to disease (Sanderson et al., 2008; Checkley et al., 2010; Taylor et al., 2010; Smith et al., 2020). Cattle at high-risk of BRD can be incubating infections that can rapidly become life-threatening despite the absence of detectable clinical

signs. Antimicrobials are often administered to these groups of high-risk cattle to eliminate or minimize BRD (Taylor et al., 2010), a practice commonly known as metaphylaxis.

Recent systematic reviews have shown that metaphylaxis or antimicrobials administered through subcutaneous injection at feedlot arrival dramatically reduce the detrimental effects of BRD (Nickell and White, 2010; O'Connor et al., 2016b, 2019; Abell et al., 2017; Word et al., 2020). Given the well documented effects of metaphylaxis on the frequency of subsequent BRD treatment, it is unclear how potential changes to AMU guidelines for disease management might affect AMR in feedlot cattle associated with disease treatment. For example, Denmark began banning the use of antimicrobials as growth promoters for finishing pigs in 2000 (Hayes and Jensen, 2014). Although the removal of antimicrobials as growth promoters significantly lowered total antimicrobial usage in Denmark, AMR has largely persisted in gut bacteria (Hayes and Jensen, 2014). Consequently, the resulting higher disease incidence has increased therapeutic usage of antimicrobials as well as overall economic losses (Hayes and Jensen, 2014).

The objective of this study is to explore the potential impact of different options for injectable metaphylactic use on the prevalence of AMR in feedlot cattle in a typical western Canadian feedlot using an agent-based model (ABM). While antimicrobials used for metaphylaxis could select for antimicrobial resistant pathogens, it is also possible that metaphylaxis may, in addition to improving animal health and welfare, reduce the use of other potentially more important classes of antimicrobials to treat BRD which are also important targets for antimicrobial stewardship initiatives.

2.3 Materials and Methods

2.3.1 Model description

A continuous-time, stochastic agent-based dynamic simulation model was constructed using AnyLogic® 8.7.4. (XJ Technologies, St. Petersburg, Russia). AnyLogic® is a Java-based simulation tool that can simulate the behaviour of agents, interactions between agents, and interactions between agents and their environment. The model simulates a typical western Canadian feedlot over a one-year period comprising 16,560 high-risk recently weaned fall placed calves per feeding cycle. Calves that have been commingled with other lots at an auction mart, transported for an extended period of time or were recently weaned with no history of vaccinations are considered to be high-risk for developing BRD (Checkley et al., 2010; Brault et

al., 2019a). Only fall placed calves at high risk of developing BRD were considered in this study. The model was used to examine the associations between different protocols for injectable metaphylactic use and the prevalence of AMR in feedlot cattle. All animals also received pulses of prophylactic in-feed chlortetracycline following metaphylaxis. Thereafter, in-feed tylosin prophylaxis followed for the duration of the feeding period, while animals were treated with antimicrobials for common illnesses, described in detail later. Each feeding cycle started on October 1st. Each feedlot pen was emptied once the pen weight reached an average of 1400 lbs at an approximate average of 320 days after arrival; individual pens could achieve market weight at various times due to variability in average daily gain (ADG) and initial arrival weights. There were four types of agents organized in a hierarchy within the model, including a feedlot made up of pens, each filled with high-risk fall placed cattle containing populations of sentinel organisms. Sentinel organisms had the potential for the presence or absence of AMR.

In the configuration used for this analysis, the feedlot included 69 pens that started with 240 calves in each pen and one chronic sick pen. Pens were described by variables, including their occupancy status and days on feed (DOF). Injectable metaphylactic AMU and in feed prophylactic AMU could be applied to each pen at specified times in the feeding period.

Calves were represented as individual agents characterized by their weight and ADG as well as their status as healthy or affected with and then treated for BRD, arthritis, or foot rot. Both weight and ADG were not affected by animal's health status (i.e., animals did not lose weight and gained at the same rate). The AMR phenotype status of two sentinel organism populations (*M. haemolytica* and generic *E. coli*) to each of 11 commonly used antimicrobials was characterized within each calf as either susceptible, stable resistant (no waning of resistance due to continued selection pressure), unstable resistant (resistance could wane over time), or intrinsically resistant.

Several simplifying assumptions were made to facilitate answering the study questions given the available data. For example, resistance was modelled at the level of drug class rather than individual drug, antimicrobials within the same drug class were assumed to have the same resistance selection and waning rates, and single organisms were modelled as sentinels for fecal commensals and respiratory pathogens of interest, with organisms selected based on longitudinal AMR data available in the literature at the time the model was constructed. Furthermore, pathways for fecal contamination with environmental transmission and direct transmission of

organisms among calves in contact were not included in the current version of the model. Finally, diseases or injuries that might result in injectable AMU other than BRD, arthritis or foot rot were not considered.

Specific model details beyond the scope of Chapter 2 are further described in Section 3.2 following the Overview, Design concepts, and Details (ODD) protocol. The following section provides a general summary of the model implemented to address questions specific to Chapter 2.

2.3.2 Process overview

The model proceeded in daily time units and was run for one-year periods representing single cohorts of cattle. At the beginning of each production year, a cohort of calves was placed in the feedlot between the start of October and mid-December. At random intervals every 1 to 3 days, between one and four loads of calves were received at the feedlot. Each load of calves (n = 240) arrived on a single day and was placed in a pen. Once all of the pens were filled, calf shipments to the feedlot ceased. After 80 to 100 days in the feedlot, calves from multiple pens were combined and resorted by weight to achieve more uniform groups for finishing and sale. Upon reaching an average pen weight of approximately 1400 lbs, the pen emptied and calves were shipped for slaughter.

Injectable metaphylaxis and in-feed prophylaxis are common management practices used in Western Canadian feedlots, particularly when calves are deemed high-risk on arrival (Checkley et al., 2010; Brault et al., 2019a). The baseline model scenario was developed to describe the selection of resistant *M. haemolytica* and generic *E. coli* where all pens of calves received both injectable metaphylaxis (tulathromycin) upon arrival and in-feed prophylaxis (chlortetracycline). *M. haemolytica* is a common bacterial agent in BRD (Callan and Garry, 2002; Cusack et al., 2003) and was used as a sentinel organism to model animal-to-animal transmission, while *E. coli* is an important bacterial agent readily isolated from cattle manure and was therefore considered a sentinel organism to model transmission from environmental reservoirs (Niu et al., 2009). Chlortetracycline was incorporated into the feed for 5 consecutive days, beginning at 18 DOF. A second 5-day pulse was delivered with a 48-hour time lapse between pulses. Subsequently, inclusion of tylosin in feed rations began on day 30 and continued until calves were shipped for slaughter.

The animal agent incorporates health and treatment status, in addition to characterizing the segregation of calves to the chronic pen that don't respond as expected to treatment protocols. A state chart regulated the health status of the calf; healthy calves could become sick and be diagnosed with either BRD, arthritis or foot rot. Subsequently, therapeutic treatment using an appropriate antimicrobial agent would be administered. If calves were treated towards the end of the finishing period, then they were held and shipped for slaughter at a later date to adhere to antimicrobial withdrawal times. All calves were assumed to be high-risk for BRD but healthy when placed in the feedlot. Thereafter, calves either remained healthy or were randomly assigned to be affected with BRD, arthritis or foot rot based on expert-reported probabilities specific for DOF and the baseline disease management protocol (metaphylaxis and in-feed antimicrobials described earlier) for high-risk calves (N. Erickson, personal communication, June 8, 2016). Affected calves were then assigned to prespecified antimicrobial treatments designated by feedlot level protocols specific for the type of illness. For BRD, the treatment protocol also took into consideration whether and how many times an animal had been previously treated. Most animals were treated and returned to their respective home pen except in the case of multiple treatment failures. After the estimated duration of selective pressure had elapsed, animals diagnosed with either arthritis or foot rot were assumed to have recovered and re-entered the healthy state. In contrast, treatment failure was possible for animals diagnosed with BRD; cattle treated for BRD could return to the healthy state or a BRD-infected state. Cattle identified for BRD treatment more than three times were sent to the chronic sick pen and stayed there for the remainder of the feeding period. A baseline risk of BRD treatment failure was assumed due to the potential for extensive disease progression before diagnosis, medication errors, and altered pharmacokinetics of antimicrobials due to pathophysiologic changes in the host animal (Booker and Lubbers, 2020). However, the probability of treatment failure for BRD also increased dynamically as an emergent outcome of the model as a direct consequence of the average pen prevalence of *M. haemolytica* resistant to the drug class used for treatment.

In this model, sentinel organism populations in each calf were subjected to selection pressure from the time of AMU to the end of the estimated duration of selective pressure. This selective pressure was triggered following AMU for metaphylaxis, in-feed AMU for prevention, and AMU for disease therapy. This selective pressure was based on a rate calibrated to match historic longitudinal AMR prevalence data collected from feedlots using comparable disease

management strategies (Benedict et al., 2015; Noyes et al., 2015) as described below in Section 2.3.4. Resistance also had the potential to wane at a rate calibrated based on historic data. Selection and waning of AMR was modelled with explicitly parallel state charts for each type of antimicrobial, which governed how selection and waning of AMR occurred within each bacterial population within each calf (Figure 2.1). Waning applied only to sentinel organisms with acquired resistance and not intrinsic resistance. Intrinsic resistance was defined as innately possessing resistance mechanisms against the respective drug class. *E. coli* contains mechanisms that confer innate resistance to macrolides and penicillins, and *M. haemolytica* possesses mechanisms that confer resistance to penicillins (Plumb, 2018; Giguère et al., 2013). All antimicrobials within the same drug class were assumed to concurrently undergo resistance selection and then waning following the cessation of AMU.

Upon arriving at the feedlot, calves might have already been colonized with resistant organisms in accordance with the probability of resistance on arrival. When an antimicrobial was administered during the feeding period at the feedlot, messages were sent to the corresponding resistance state charts. A susceptible bacterial population within a calf could become resistant as prescribed by the rate of AMR selection with respect to specific class of antimicrobial. Within that bacterial population, AMR for that particular class of antimicrobial was assumed to wane once selective pressure from AMU stopped. The estimated duration of selective pressure was assumed to be based on the half-life of the drug (Table 2.3). Selection and waning of AMR was assumed to occur uniformly across the drug class, such that once an antimicrobial was administered, resistance to other antimicrobials within the same drug class would likewise undergo selection and waning.

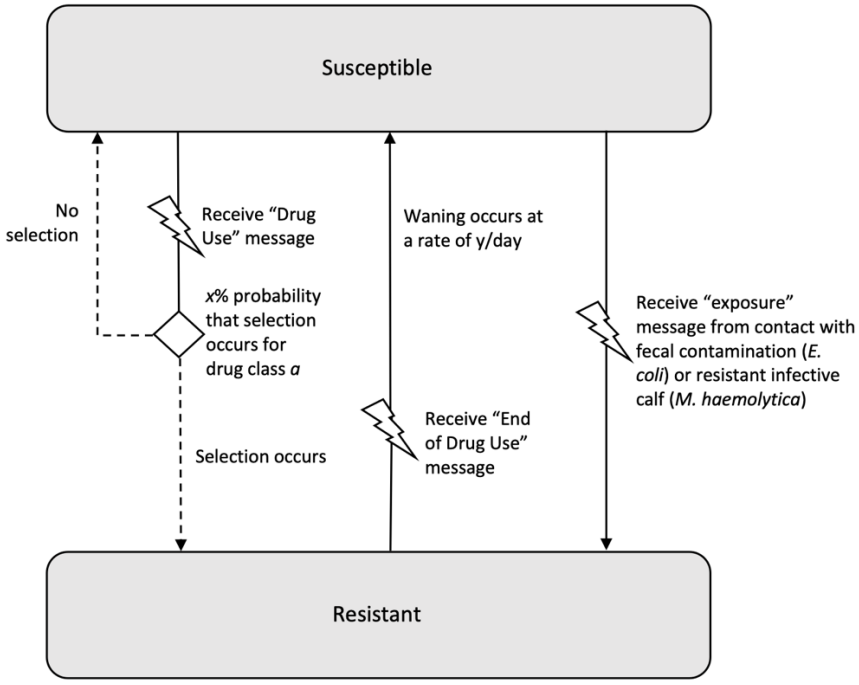

Figure 2.1: Process overview of AMR in the ABM. Diamonds represent chance outcomes; dotted lines represent options that could occur given a true or false outcome. Lightning bolts indicate the sending or receiving of messages to/from other agents (i.e., pen, animal, organism). The selection probabilities for resistance are different and specific to a particular drug class and type of sentinel organism (e.g., *M. haemolytica* or *E. coli*).

2.3.3 Design concepts

Basic principle. Under the selective pressure of an antimicrobial agent, microorganisms may acquire resistance that allow them to survive in the presence of antimicrobials and outcompete susceptible microorganisms.

Emergence. Antimicrobial resistance and the resulting probability of treatment failure for BRD with corresponding impacts on need for additional AMU emerge dynamically with resulting AMR in the model. Selection for and waning of AMR in feedlot calves occurs during the entire feeding period in response to different types of pressure from metaphylactic, in-feed and therapeutic AMU. In addition to providing selection pressure for resistance to the antimicrobial agent being used, metaphylaxis is expected to influence the frequency and types of antimicrobials used for treatment of BRD. The result is emergent AMR patterns that are difficult to predict. This relationship is made more complex by the potential for AMU in response to treatment failure to further increase AMR and subsequent AMU.

Stochasticity. All disease occurred at rates associated with an exponential distribution of residence time, with the rate representing a probability per time unit, or in this case per day. Incidence of first treatment for BRD is governed by rates specific to DOF. Incidence rates of both arthritis and foot rot were randomly selected from predefined ranges derived from observations reported by an industry expert (N. Erickson, personal communication, June 8, 2016). Further, selection and waning of AMR were based on a rate calibrated from historical data (Noyes et al., 2015; Benedict et al., 2015).

Collectives. The model is organized as a hierarchy. The feedlot contains the aggregation of individual pens, each pen has a specific group of calves assigned at the time of arrival, and each calf has their own sentinel organisms.

Observation. Mean resistance prevalence values of *E. coli* and *M. haemolytica* for all feedlot pens were calculated daily and updated graphically. For *E. coli*, the reported drug classes were cephalosporins, fluroquinolones, trimethoprim, sulfonamides, phenicols and tetracyclines. The same drug classes were reported for *M. haemolytica*, with the addition of macrolides. Drugs to which the bacteria are considered to be intrinsically resistant were not reported. Occurrence of respiratory disease, arthritis, and foot rot treatments as well as the BRD chronic case incidence rate were also reported.

2.3.4 Details

Initialization

The model is initialized as an empty feedlot. Each production cycle starts as the feedlot pens begin to fill on the 1st of October.

Input

Parameter inputs were obtained from the peer-reviewed literature and discussions with feedlot industry experts to customize the model for typical practices in western Canada (Table 2.1). Herd management practices and antimicrobial treatment choices used in the baseline scenario are provided in Table 2.1. A five-year retrospective feedlot study was referenced for first treatment incidences in fall calves receiving tulathromycin and tildipirosin (N. Erickson, personal communication, June 8, 2016). In this study, first treatment incidence averaged 0.06% at day 5 on feed, peaked at 1.14% by day 25 and stabilized to between 0.05 and 0.10% from days 60 to 100.

Table 2.1: Values used to parameterize the feedlot and animal management in the ABM.

Parameter	Value
Number of animals per pen	240
Allow metaphylaxis	True
Allow in-feed prophylaxis	True
DOF to begin first round of in-feed prophylaxis (chlortetracycline)	18th day on feed
Duration of in-feed prophylactic use	5 days
DOF to begin second round of in-feed prophylaxis (chlortetracycline)	25th day on feed
DOF to begin tylosin in-feed prophylaxis	30th day on feed
Product used for injectable metaphylaxis	Tulathromycin
BRD first treatment incidences	Frequency distribution of first treatment for BRD specific to DOF from 5-year retrospective analysis (unpublished data); Cumulative incidence: 26%
Baseline probability of failure for first BRD treatment	Selected from uniformly distributed double value between 10 and 25% or relative frequency of AMR if greater than baseline value of treatment failure for first BRD treatment option
Baseline probability of failure for second BRD treatment	50% or relative frequency of AMR if greater than baseline value of treatment failure for second BRD treatment option
Baseline probability of failure for third BRD treatment and movement to the chronic pen	Relative frequency of AMR
Arthritis incidence	Selected from a uniform distribution between 0.6 and 1.6% per week from days 30 to 100 on feed; Cumulative incidence: 1.1%
Foot rot incidence	Selected from a uniform distribution between 2 and 12% per week over 245 days during the feeding period; Cumulative incidence: 6.0%

Reference: N. Erickson, personal communication, June 24, 2016

The antimicrobial agents used for therapeutic disease treatment in the model were listed in Table 2.2. A variation option in the model allowed for the selection of either a default treatment option or random treatment selection from a list of commonly used antimicrobial agents (N. Erickson, personal communication, June 8, 2016). For example, when calves were not successfully treated with florfenicol as the first BRD therapeutic treatment, two additional antimicrobial agents (ceftiofur or enrofloxacin) were listed as alternative second treatment

options. One of these two antimicrobials was randomly selected with equal probability at model start-up and used as the second therapeutic treatment for BRD if the variation option was selected. Trimethoprim sulfadoxine was listed as the therapeutic choice for third BRD treatments. Calves treated a third time and that did not respond were sent to the chronic pen for the remainder of the feeding period.

For arthritis, tulathromycin, florfenicol and oxytetracycline were selected as common therapeutic treatments for Western Canadian feedlots. For each simulation run, one of these three antimicrobials were selected at random with uniform probability upon start-up as the treatment option for arthritis. One of ceftiofur or penicillin were randomly selected at startup for treatment of foot rot.

Table 2.2: Treatment options for BRD, Arthritis, and Foot Rot used in the model.

	Notes
BRD	
1st treatment	Florfenicol.
2nd treatment	Ceftiofur or enrofloxacin (randomly chosen at the feedlot-level at start up).
3rd treatment	TMS
Arthritis	Treated once using tulathromycin, florfenicol, or oxytetracycline (randomly chosen with equal probability at the feedlot-level at start up for each simulation run).
Foot rot	Treated once using ceftiofur or penicillin (randomly chosen with equal probability at the feedlot-level at start up for each simulation run).

Reference: N. Erickson, personal communication, June 24, 2016

Eleven antimicrobials were considered in the present version of the model, and were subdivided into classes (Table 2.3). Some antimicrobials (e.g., TMS) belong to multiple drug classes and were included in each relevant class.

Elimination half-lives determined the time for a drug to be eliminated from the body once drug administration was discontinued. Most drugs are eliminated by 3 to 5 half-lives (Merck Manual, 2015). Since the period where selective pressure was being exerted on microbial communities was not available for these products, three half-lives was chosen as a conservative measure of effective duration of selective pressure. This was consistent with the available published withdrawal times. These values could be modified to account for alternative hypotheses if appropriate data were available.

Table 2.3: Antimicrobials included within the model.

Category/Class	Antimicrobial	Withdrawal Time (days)	Estimated Effective Duration of Selective Pressure (days)*
Category I[1]			
Cephalosporins (3rd & 4th generation)[1]	Ceftiofur crystalline free acid	13	7.8
	Ceftiofur hydrocholoride	3	4.3
Fluoroquinolones	Enrofloxacin	36	0.8
Category II			
Macrolides[1,3]	Tulathromycin	44	8.3
	Tylosin	0	0.1
Trimethoprim[2,3]	Trimethoprim/Sulfonamide	10	1.4
Penicillins[1,5]	Penicillin	5	2.3
Category III			
Phenicols[1]	Florfenicol	55	6.7
	Florfenicol/Flunixin	60	6.7
Tetracyclines[1,4,6]	Oxytetracycline	48	2.7
	Chlortetracycline	5	2.0
Sulfonamides[1,3]	Trimethoprim/Sulfonamide	10	1.4

[1]Bayer Corporation, 1991
[2]Riviere et al., 2003
[3]Plumb, 2018
[4]Toutain and Raynaud, 1983
[5]Papich et al., 1993
[6]Reinbold et al., 2010
*Effective duration of selective pressure was estimated using the half-life of each antimicrobial × 3.

On-arrival resistance prevalence to each drug class for *M. haemolytica* (Noyes et al., 2015) and *E. coli* (Benedict et al., 2015) were obtained from respiratory and fecal samples collected between 2007 and 2010 as part of a large surveillance project conducted in western Canada. The study authors supplied original data to allow calibration of antimicrobial agent and organism specific selection and waning rates to best fit reported resistance prevalence at day 45, 90, 135, 180 and 225 from 2007 – 2008 (P. Morley, personal communication, 2018). Antimicrobials within the same class were assumed to possess the same susceptibility to resistance mechanisms and to have the same selection and waning rates. However, the model can

readily accommodate modifications to this assumption should more product specific data become available.

An automated optimization and calibration experiment was conducted to determine the selection and waning rates for each drug class assuming, for this specific scenario, that drug use was the only factor contributing to AMR. Calibration varied selection and waning rates over continuous ranges to minimize the objective function quantifying the discrepancy between the model AMR prevalence output and empirical AMR prevalence data. The model was run for a minimum of 10,000 iterations, with each iteration representing a one-year feeding period. Further, each iteration was run for minimum of six replications to allow for stochastic variation within a single parameter set, when the 95% confidence level was reached. The confidence level was fixed at 95%, constructed around the mean of replication results (i.e., the objective function values), and a relative error of 5%. If the 95% confidence level was not met, maximum number of 10 replications were completed. The resulting calibrated selection and waning rates for both *M. haemolytica* and *E. coli* are presented in Table 2.4.

Table 2.4: Calibrated selection and waning rates for *E. coli* and *M. haemolytica* based on reported resistance prevalence for drug classes from previous publications[1,2].

Drug Class	*Selection Rate*[5]	*Waning Rate*[5]
M. haemolytica[1]		
Cephalosporin	0.110	18.5
Fluoroquinolone	0.760	0.003
Macrolide	0.004	47.0
Penicillin[3,4]	Intrinsically resistant	
Phenicol	0.072	43.2
Sulfonamide	0.053	0.009
Tetracycline	0.015	0.232
Trimethoprim	0.997	0.097
E. coli[2]		
Cephalosporin	0.613	42.6
Fluoroquinolone	0.043	0.015
Macrolide[3,4]	Intrinsically resistant	
Penicillin[3,4]	Intrinsically resistant	
Phenicol	0.195	0.000
Sulfonamide	0.994	0.000
Tetracycline	0.353	0.000
Trimethoprim	0.765	0.008

[1]Noyes et al., 2015; [2]Benedict et al., 2015; [3]Plumb, 2018; [4]Giguère et al., 2013
[5]Selection and waning rates are reported as the mean probability of the event occurrence per day. For values greater than 1, the event will on average occur within a smaller time unit than once per day.

2.3.5 Simulation experiments

The optimization and calibration experiment was used to parameterize the model. The ABM is stochastic and each model run, simulation or iteration potentially produces a different outcome. As a result, generating simulation outputs also required Monte Carlo experiments; these were also important when drawing parameter values from distributions. A series of such experiments were conducted to obtain a collection of simulation outputs with predicted resistance prevalence at various timepoints through the feeding period for specific antimicrobials of interest. Each experimental scenario was run for 10,000 iterations with 5 replications (realizations) for each iteration to allow for stochastics within specific parameter values selected at the start of each iteration or model run. The resulting distributions of AMR prevalence were compared among the targeted intervention protocols at each time point.

The impact of three injectable metaphylaxis protocols (tulathromycin (baseline), oxytetracycline, and no on-arrival metaphylaxis) were compared by measuring resulting

prevalence on days 20, 90, and 225 of resistance to three examples of products used in treating clinical BRD: oxytetracycline, florfenicol, and TMS. Resistance prevalence on day 20 was compared to assess the effect of metaphylaxis choices on AMR expected to impact treatment choices for BRD. Selection of resistant bacteria due to the administration of metaphylaxis on arrival has been reported for the period prior to day 20 (Woolums et al., 2018). The peak of observed resistance in our baseline data (Noyes et al., 2015; Benedict et al., 2015) occurred near day 90 for most of the drug classes and coincided with the approximate mid-point of the feeding period. Both 20 and 90 DOF are times that have implications for animal health and welfare since AMR can make antimicrobials less effective at treating bacterial diseases during the feeding period and contribute to treatment failure, resulting in a higher morbidity and mortality. Treatment protocols were also compared on day 225 as animals approached market weight. The presence of resistance at time of slaughter is important due to the potential for resistant pathogens to enter the human food chain.

The three drug classes evaluated for development of resistance were chosen based on respective resistance prevalence from available literature sources for *M. haemolytica* (Noyes et al., 2015) and *E. coli* (Benedict et al., 2015) and potential to be influenced by choice of metaphylaxis protocol. Both *M. haemolytica* and *E. coli* exhibited the highest and most consistent resistance to the florfenicol, oxytetracycline and TMS classes in the available literature. In addition, common therapeutic treatments for BRD represented in the model included florfenicol and TMS. In-feed chlortetracycline was utilized in the model to prevent *Histophilus somni*-associated disease, including respiratory disease, arthritis, and sudden death, and injectable oxytetracycline was also used for treating arthritis in some scenarios. Currently, macrolides used for metaphylaxis in feedlot cattle includes tilmicosin, tulathromycin, gamithromycin and tildipirosin, which were approved in 1990, 2007, 2010, and 2012, respectively (Brault et al., 2019a; Bergen, 2020). Although macrolides have become more commonly used in the management of BRD, many were newer products at the time the reference AMR prevalence used in this study was collected (2007 – 2008), and the observed prevalence of AMR to macrolides at that time was relatively low in *M. haemolytica*. As a result, the data available for this model did not allow us to effectively evaluate the impact of metaphylaxis on macrolide resistance in *M. haemolytica*. Additionally, *E. coli* was not considered a good sentinel organism for macrolide resistance (Plumb, 2018). The Canadian Integrated Program for

Antimicrobial Resistance Surveillance (CIPARS) has limited data on resistant *E. coli* in feedlot cattle for selected groups of antimicrobial agents. However, longitudinal studies similar to the research conducted by Noyes et al. (2015) and Benedict et al. (2015) have not been repeated.

Baseline scenario. The baseline scenario was developed to describe typical management practices in a Western Canadian feedlot for fall placed calves considered at high-risk for BRD. This scenario was intended to resemble conditions consistent with the AMR and BRD data collected and used for calibration (Benedict et al., 2015; Noyes et al., 2015; N. Erickson, personal communication, June 8, 2016; P. Morley, personal communication, 2018). Mean BRD incidence rates used in the baseline scenario were from a five-year retrospective study calculated from high-risk calves receiving macrolides as on-arrival injectable metaphylaxis (N. Erickson, personal communication, June 8, 2016).

Alternate metaphylaxis scenario. The same management practices from the baseline scenario described above were employed, except that oxytetracycline was administered on arrival as metaphylaxis. A recent study by O'Connor et al. (2019) determined the comparative efficacy of antimicrobials used to control BRD in feedlot cattle. The comparative risk ratio for developing BRD with oxytetracycline metaphylaxis compared to tulathromycin metaphylaxis was used to adjust the expected baseline incidence rate of BRD. Specifically, a value was drawn at the start of each simulation run from the triangular distribution representing the reported risk ratio and 95% CI with minimum value (1.43), maximum value (5.26), and most likely value (2.56). For each simulation run, a value drawn from the distribution was then multiplied by the baseline BRD incidence rate to achieve an adjusted BRD incidence rate expected following oxytetracycline metaphylaxis.

No metaphylaxis scenario. For the no metaphylaxis intervention, the comparative risk ratio for no metaphylaxis compared to tulathromycin metaphylaxis (O'Connor et al., 2019) was used to adjust the BRD incidence rate. The comparative risk ratio for no antimicrobial compared to tulathromycin was represented using a triangular distribution with a minimum value of 2.04, maximum value of 8.18, and most likely value of 4.79.

2.3.6 Statistical analysis

The normality assumption from the outputted resistance prevalence was evaluated using histograms, normal quantile plots and the Shapiro-Wilk test. Since the normality assumption was

violated, the non-parametric Kruskal-Wallis test was used, followed by Wilcoxon rank sum test to compare post-hoc differences in the sum of ranks for specific pairwise comparisons between results for each scenario. Differences were considered significant when $P \leq 0.05$. All analyses were conducted using Stata 15 (StataCorp LLC, College Station, TX).

2.4 Results

Escherichia coli

The prevalence of resistance to florfenicol, TMS, and oxytetracycline are presented below for *E. coli* (Table 2.5, Figures 2.2, 2.3, and 2.4, respectively).

Table 2.5: Mean (± standard deviation) antimicrobial resistant *E. coli* prevalence (proportion of resistant animals in the total population) on days 20, 90, and 225 following three scenarios for injectable metaphylaxis administration on-arrival.

	Metaphylaxis		
Drug	Tulathromycin	Oxytetracycline	No metaphylaxis
Florfenicol			
Day 20	0.022 ± 0.002	0.028 ± 0.003	0.033 ± 0.004
Day 90	0.064 ± 0.002	0.125 ± 0.003	0.157 ± 0.003
Day 225	0.067 ± 0.002	0.130 ± 0.003	0.162 ± 0.003
TMS			
Day 20	0.006 ± 0.001	0.006 ± 0.001	0.006 ± 0.001
Day 90	0.020 ± 0.001	0.069 ± 0.002	0.118 ± 0.003
Day 225	0.008 ± 0.001	0.029 ± 0.002	0.049 ± 0.003
Oxytetracycline			
Day 20	0.269 ± 0.030	0.536 ± 0.025	0.269 ± 0.030
Day 90	0.687 ± 0.004	0.804 ± 0.003	0.686 ± 0.004
Day 225	0.687 ± 0.004	0.804 ± 0.003	0.687 ± 0.004

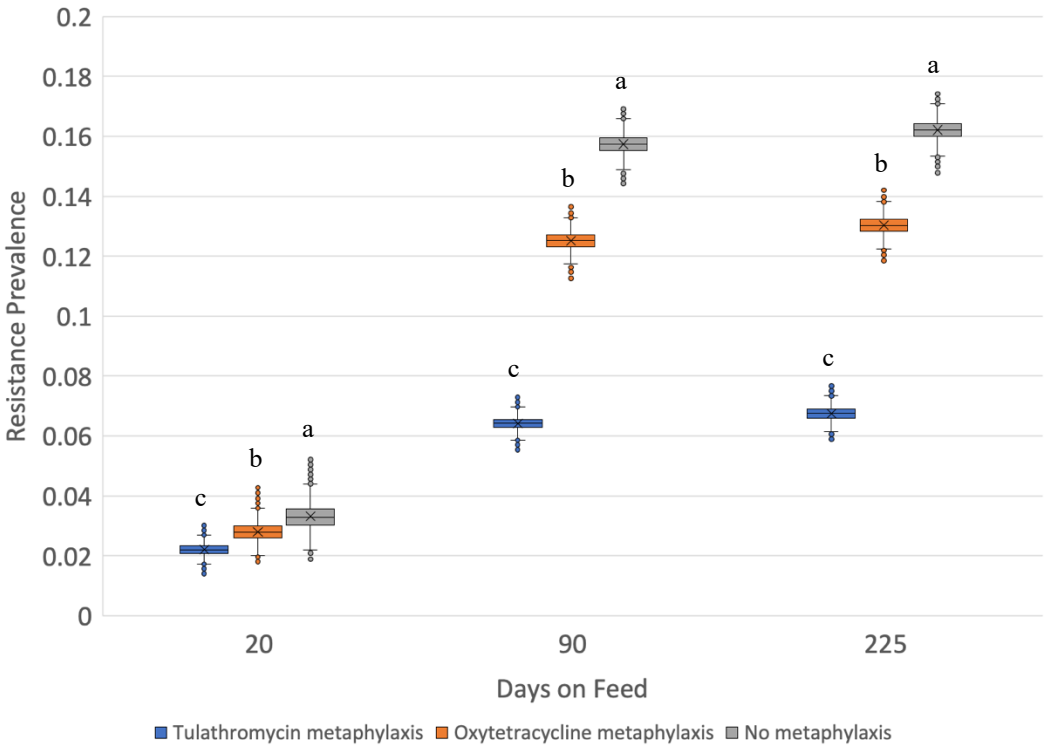

Figure 2.2: Prevalence of resistance to florfenicol in *E. coli* among different metaphylaxis protocols at 20, 90, and 225 DOF. Median resistance prevalence values with different letters differ significantly among treatment protocols ($P < 0.05$).

Florfenicol resistance prevalence in *E. coli* was significantly different between treatment protocols on days 20, 90, and 225 ($P < 0.0001$). While statistically significant, the differences among resistance to florfenicol at day 20 were relatively small. Resistance prevalence was 0.022, 0.028, 0.033 for tulathromycin, oxytetracycline, and no metaphylaxis on day 20, respectively, and increased to 0.067, 0.130, 0.162 on day 225, respectively (Table 2.5). Resistance was greatest when no injectable metaphylaxis was used on arrival, while tulathromycin metaphylaxis resulted in the lowest resistance prevalence.

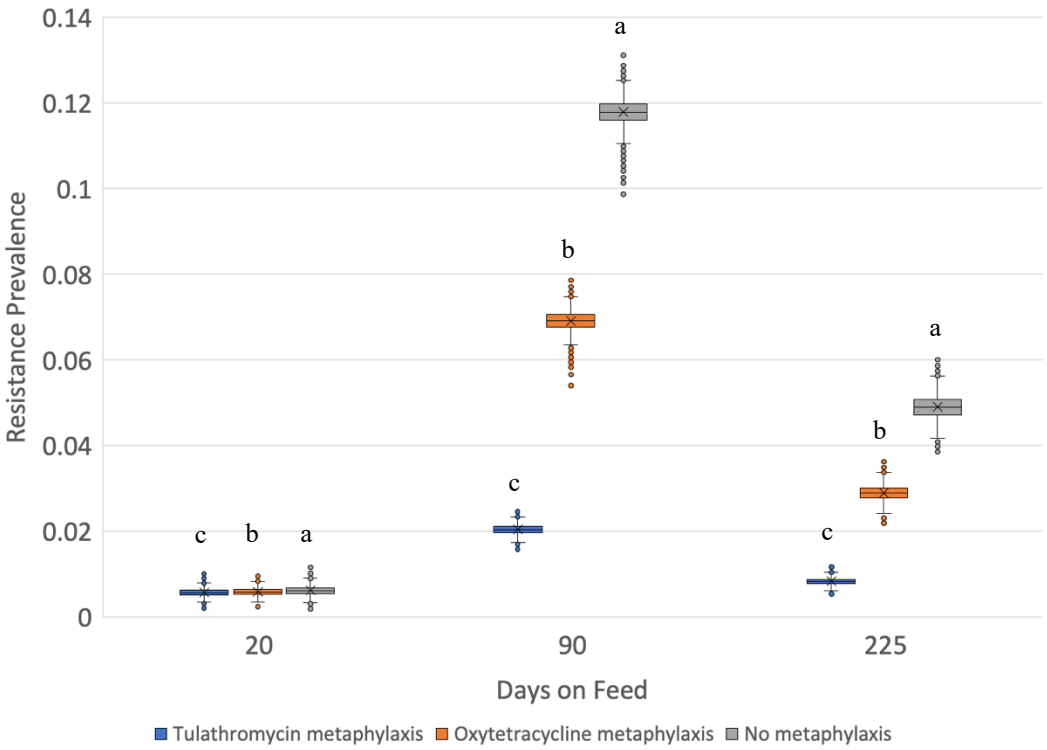

Figure 2.3: Prevalence of resistance to TMS in *E. coli* among different metaphylaxis protocols at 20, 90, and 225 DOF. Median resistance prevalence values with different letters differ significantly among treatment protocols ($P < 0.05$).

The prevalence of TMS resistant *E. coli* significantly differed between treatment protocols on days 20, 90, and 225 ($P < 0.0001$) with the control group having the highest resistance value and the tulathromycin metaphylaxis group having the lowest. While the differences at day 20 were statistically significant, the magnitude of the differences among metaphylaxis protocols were very small. Prevalence of resistance to TMS in pens that received tulathromycin injectable on-arrival was 0.006, 0.020, 0.008 on days 20, 90, and 225, respectively. Mean prevalence in pens that received oxytetracycline was 0.006, 0.069, 0.029 on days 20, 90, and 225, respectively. Finally, prevalence was 0.006, 0.118, 0.049 on days 20, 90, and 225, respectively, in pens that received no metaphylaxis on-arrival.

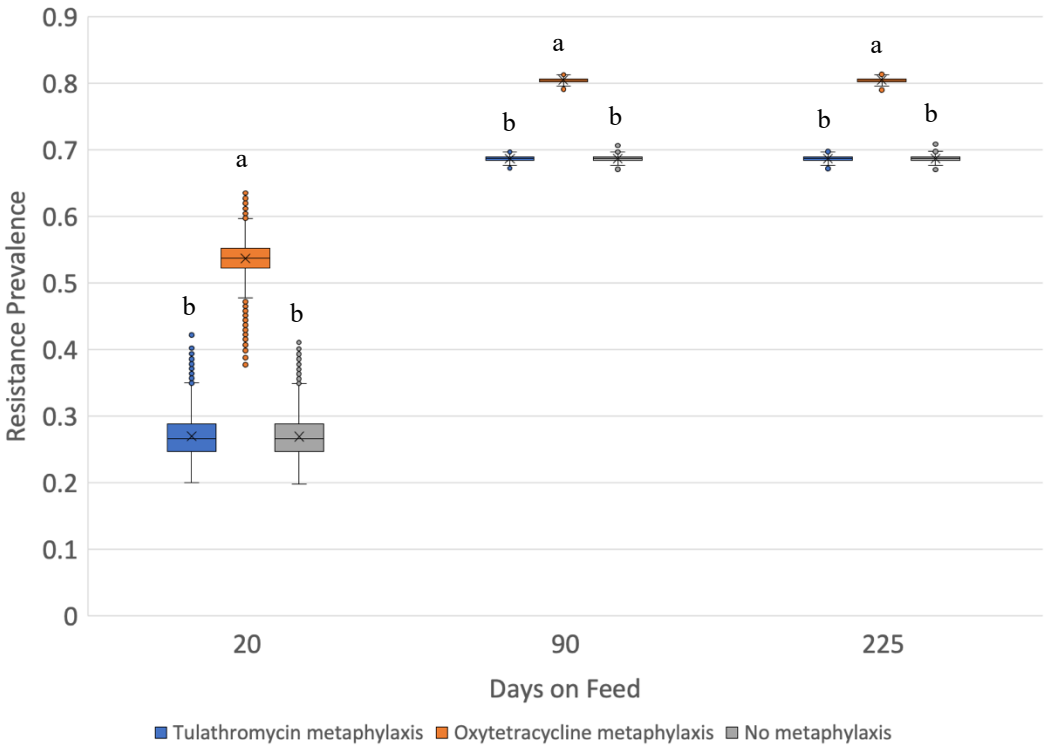

Figure 2.4: Prevalence of resistance to oxytetracycline in *E. coli* among different metaphylaxis protocols at 20, 90, and 225 DOF. Median resistance prevalence values with different letters differ significantly among treatment protocols ($P < 0.05$).

Resistance prevalence for oxytetracycline resistant *E. coli* was greatest when oxytetracycline was used on arrival as metaphylaxis and significantly differed from both tulathromycin and control treatment protocols on days 20, 90, and 225 ($P < 0.0001$). Resistance prevalence to oxytetracycline was 0.269, 0.536, 0.269 on days 20 for pens that received tulathromycin on arrival, oxytetracycline on arrival and no metaphylaxis, respectively, and increased to 0.687, 0.804, 0.687 on day 225 for the tulathromycin, oxytetracycline and no metaphylaxis treatments, respectively. No significant differences were observed between tulathromycin and control treatments on days 20 ($P = 0.29$), 90 ($P = 0.10$), and 225 ($P = 0.10$).

For florfenicol and oxytetracycline, resistance prevalence was lowest on day 20 and highest on day 225 for each treatment protocol. Prevalence for TMS-resistant bacteria was lowest on day 20 and highest on day 90. Resistance prevalence persisted through the feeding period for both florfenicol and oxytetracycline, however TMS resistance prevalence decreased gradually after peak resistance prevalence observed at day 90.

Mannheimia haemolytica

Resistance prevalence to florfenicol, TMS, and oxytetracycline is presented below for *M. haemolytica* (Table 2.6, Figures 2.5, 2.6, and 2.7, respectively).

Table 2.6: Mean (± standard deviation) antimicrobial resistant *M. haemolytica* prevalence (proportion of resistant animals in the total population) on days 20, 90, and 225 following injectable metaphylaxis administration on-arrival.

	Metaphylaxis		
Drug	Tulathromycin	Oxytetracycline	No metaphylaxis
Florfenicol			
Day 20	0.001 ± 0.0004	0.003 ± 0.001	0.004 ± 0.001
Day 90	$3.6 \times 10^{-4} \pm 1.6 \times 10^{-4}$	0.001 ± 0.0002	0.001 ± 0.0002
Day 225	0.000	0.000	0.000
TMS			
Day 20	$1.2 \times 10^{-4} \pm 1.7 \times 10^{-4}$	$4.1 \times 10^{-4} \pm 4.8 \times 10^{-4}$	$7.2 \times 10^{-4} \pm 8.2 \times 10^{-4}$
Day 90	0.004 ± 0.001	0.017 ± 0.003	0.031 ± 0.006
Day 225	$1.8 \times 10^{-7} \pm 3.4 \times 10^{-6}$	$7.8 \times 10^{-7} \pm 7.1 \times 10^{-6}$	$1.4 \times 10^{-6} \pm 9.6 \times 10^{-6}$
Oxytetracycline			
Day 20	0.030 ± 0.002	0.044 ± 0.003	0.030 ± 0.002
Day 90	0.048 ± 0.002	0.063 ± 0.002	0.048 ± 0.002
Day 225	0.033 ± 0.001	0.051 ± 0.002	0.033 ± 0.002

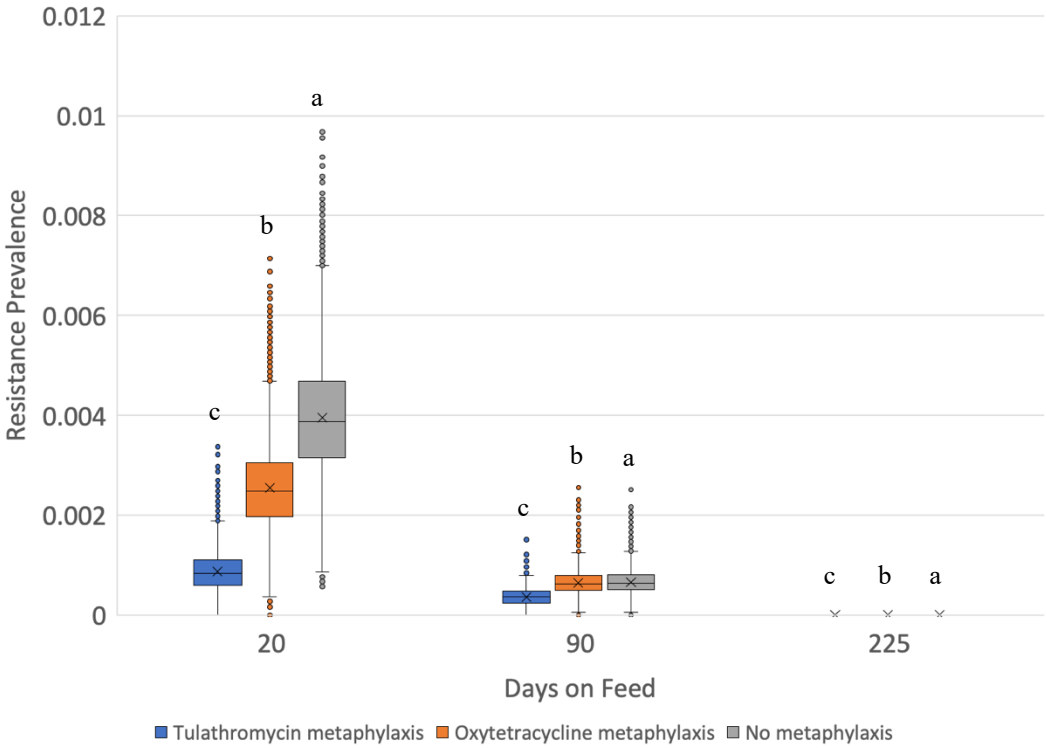

Figure 2.5: Prevalence of resistance to florfenicol in *M. haemolytica* among different metaphylaxis protocols at 20, 90, and 225 DOF. Median resistance prevalence values with different letters differ significantly among treatment protocols ($P < 0.05$).

Predicted prevalence of florfenicol resistance was less than 1% across all treatments and as such the magnitude of overall differences among all treatment protocols were small. Florfenicol resistant *M. haemolytica* did, however, significantly differ between tulathromycin (0.001), oxytetracycline (0.003) and no metaphylaxis (0.004) on day 20 ($P < 0.0001$). Further, resistance prevalence was 3.6×10^{-4}, 0.00064 and 0.00066 for tulathromycin, oxytetracycline, and no metaphylaxis on day 90, respectively ($P < 0.0001$). The control treatment protocol exhibited the greatest resistance prevalence while tulathromycin had the lowest. However, there was no difference between treatment protocols on day 225.

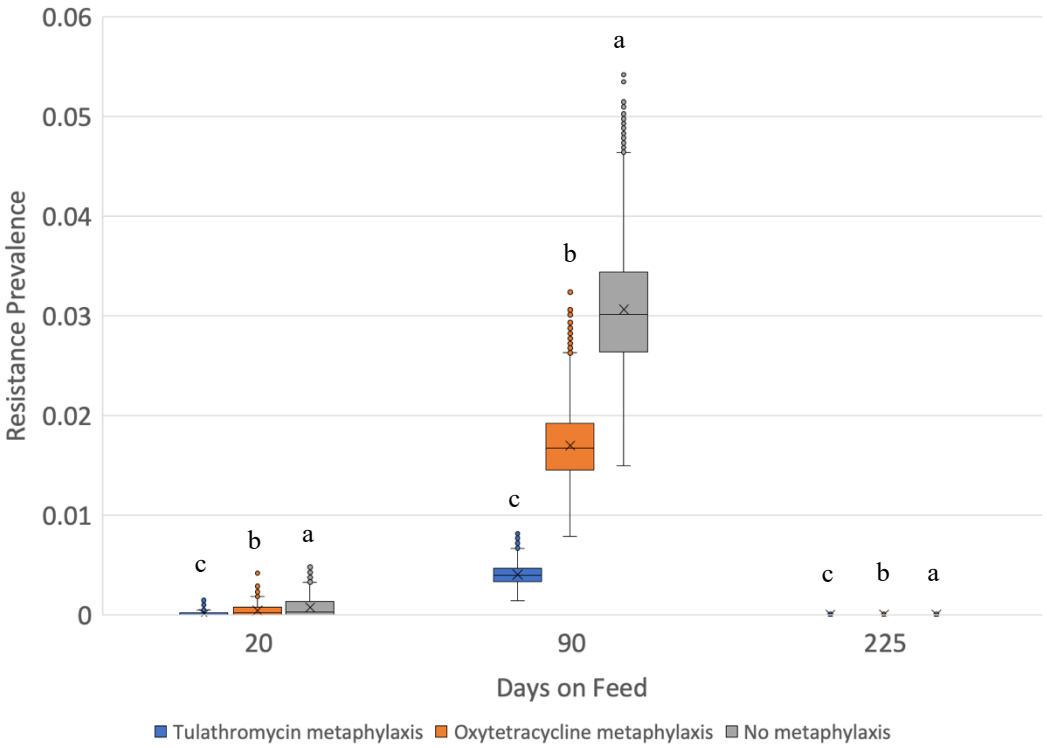

Figure 2.6: Prevalence of resistance to TMS in *M. haemolytica* among different metaphylaxis protocols at 20, 90, and 225 DOF. Median resistance prevalence values with different letters differ significantly among treatment protocols ($P < 0.05$).

The prevalence of resistance to TMS varied slightly more than florfenicol during the study and among metaphylaxis protocols. There were significant differences between all three treatment protocols for TMS resistance on days 20, 90, and 225 ($P < 0.0001$), with the control group having the highest resistance value and tulathromycin having the lowest. Resistance prevalence to TMS for the tulathromycin (1.8×10^{-7}), oxytetracycline (7.8×10^{-7}) and no metaphylaxis (1.4×10^{-6}) on day 225 was the lowest, while prevalence values peaked on day 90 to 0.004, 0.017 and 0.031, respectively. Day 20 resistance prevalence values were intermediate at 1.2×10^{-4}, 4.1×10^{-4} and 7.2×10^{-4} for tulathromycin, oxytetracycline and no metaphylaxis, respectively. Despite significant differences between metaphylaxis protocols on day 225, the numerical magnitude of the differences among each scenario was very small.

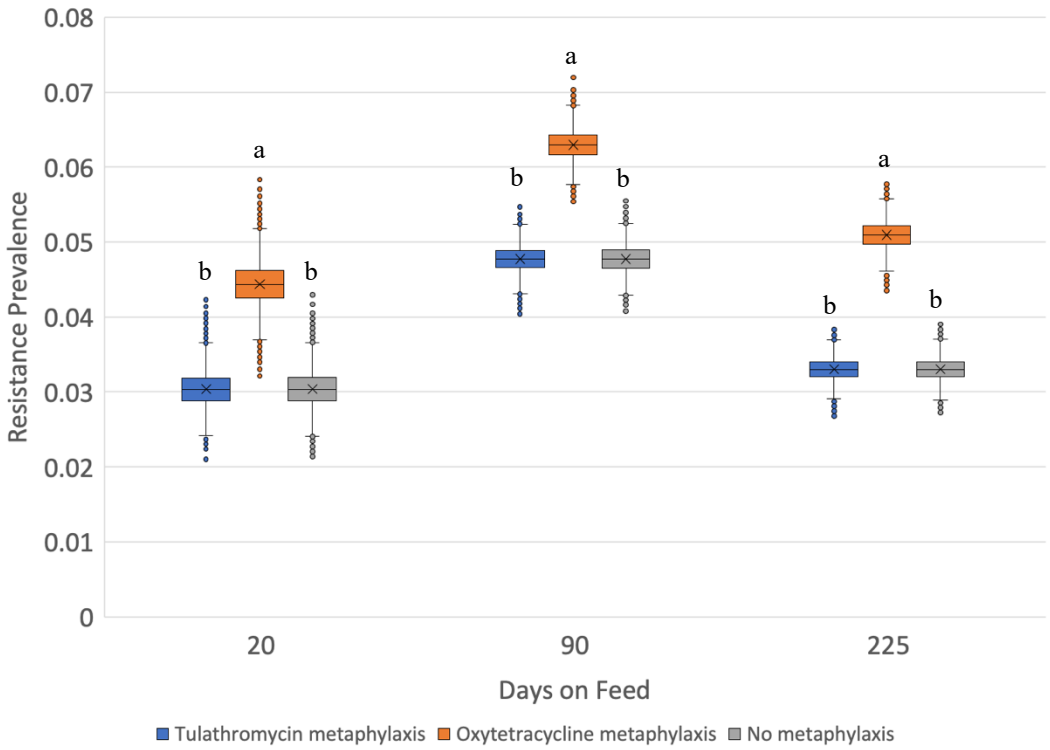

Figure 2.7: Prevalence of resistance to oxytetracycline in *M. haemolytica* among different metaphylaxis protocols at 20, 90, and 225 DOF. Median resistance prevalence values with different letters differ significantly among treatment protocols ($P < 0.05$).

Similar to *E. coli*, oxytetracycline resistance for *M. haemolytica* was significantly greater for the oxytetracycline metaphylaxis group than the tulathromycin and control groups on days 20, 90, and 225 ($P < 0.0001$); no differences were observed between tulathromycin and control treatment on days 20 ($P = 0.48$), 90 ($P = 0.65$), and 225 ($P = 0.84$). Resistance prevalence associated with oxytetracycline were 0.030, 0.044, and 0.030 on day 20 for pens that received tulathromycin, oxytetracycline and no metaphylaxis, respectively. These values increased to 0.033, 0.051 and 0.033 by day 225 for the tulathromycin, oxytetracycline and no metaphylaxis treatments, respectively.

Resistance prevalence for florfenicol was greatest on day 20 and lowest on day 225 for all treatment protocols. For TMS and oxytetracycline, the lowest resistance prevalence was observed on days 225 and 20, respectively; the highest resistance prevalence was observed on day 90 for both drug classes across all treatment protocols. For all three antimicrobials, resistance prevalence decreased gradually after peak resistance prevalence observed at day 90.

2.5 Discussion

In addition to the natural evolution of microorganisms over time, widespread use of antimicrobial agents in feedlot cattle increases potential selection pressure for the emergence of AMR (Timsit et al., 2017; Holman et al., 2018). It is important to understand the factors that contribute to AMR emergence to adequately address the threat. Metaphylactic treatment is often criticized for providing the basis of selection of AMR in feedlot cattle. However, the effects of decreased metaphylactic AMU in feedlot cattle on AMR have not been systemically explored. The objective of the current study was to investigate the influence of on-arrival injectable metaphylaxis on AMR, assuming all AMR emergence in the feedlot was a result of exposure to AMU. This was achieved using a stochastic agent-based dynamic simulation model to describe resistance prevalence in *E. coli* and *M. haemolytica* during the feeding period for calves receiving tulathromycin, oxytetracycline or no metaphylaxis upon arrival at the feedlot.

The drug classes evaluated in this study were chosen based on resistance prevalence values from available literature sources for both *M. haemolytica* and *E. coli* at the time of model development. Since each of these organisms exhibited the highest and most consistent resistance to the florfenicol and TMS classes, and these antimicrobials were used for BRD therapy, evaluation of these two antimicrobials provided the best potential for influence by choice of metaphylaxis protocol in the model. Macrolides are the primary antimicrobials administered to high-risk cattle (Brault et al., 2019b). However, prevalence of macrolide-resistant *M. haemolytica* has only recently increased in conjunction with increased macrolide use. Resistance prevalence was low for macrolides as reported by Noyes et al. (2015) from feedlot cattle samples collected in 2007 and 2008. For nasopharyngeal samples collected on arrival and prior to antimicrobials being administered at the feedlot, Andrés-Lasheras et al. (2021) found high macrolide minimum inhibitory concentrations in only *Mycoplasma bovis* when investigating BRD complex bacterial members. The authors also reported low prevalence for tilmicosin (<7%) and tulathromycin (<5%) resistant *Histophilus somni*, *Pasteurella multocida* and *M. haemolytica*. While recent AMR research has indicated high resistance values to tulathromycin (71.8%) and tilmicosin (79.5%) at the time of treatment for BRD in both healthy (n = 210) and sick animals (n = 2017) sampled at four feedlots from November 2015 to January 2016 (Timsit et al., 2017), this data was not available in the longitudinal form required for the current study. Thus, macrolide resistance was excluded for consideration at the time of this thesis.

The results from this analysis suggest that the resistance prevalence for florfenicol and TMS were greater for the no-metaphylaxis scenario when compared to metaphylaxis with tulathromycin and oxytetracycline. Further, the resistance prevalence for florfenicol and TMS were lower when tulathromycin was used as metaphylaxis in contrast to oxytetracycline. While the differences were statistically significant, the magnitude of the differences for resistance in *M. haemolytica* were small. The differences observed in resistance for *E. coli* were more substantial. The observed differences in AMR associated with AMU wasn't unexpected given the choice to leverage treatment data from the network meta-analysis from O'Connor et al. (2019) which reported expected differences and 95% CI among first treatment rates following different metaphylaxis protocols. This was further supported by Booker et al. (2007), who observed lower treatment and relapse rates in feedlot calves treated with tulathromycin metaphylactic treatment compared to oxytetracycline. Further, Szasz et al. (2019) observed an additive positive effect on decreased morbidity in cattle receiving tulathromycin and in-feed chlortetracycline compared with those receiving tulathromycin alone. In contrast, there were no differences in performance when administering metaphylactic tulathromycin concurrent with in-feed chlortetracycline by Wallace et al. (2009). However, timing and treatment doses of the in-feed chlortetracycline may have contributed to the inconsistent results between these studies.

Oxytetracycline resistance was expected to increase due to selective pressure when used as on-arrival metaphylaxis. However, there were no drugs from the tetracycline class used as BRD therapeutic treatment in this model. Therefore, any reductions to the incidence of BRD would not result in a corresponding decrease for therapeutic oxytetracycline use. Subsequently, no downstream effects or differences between tulathromycin or no metaphylaxis protocols were observed. Prolonged in-feed chlortetracycline use further influences the selection of resistance for tetracycline. As a result, tetracycline had the greatest resistance prevalence following oxytetracycline metaphylaxis, rather than the control group as observed in the other drug classes.

These observations are valuable since there are differences in the relative importance to human health of drug classes used in the model. Some drug classes are considered more important than others in the treatment of serious human medicine bacterial infections, and resistance selection of those antimicrobials from the beef industry could have serious public health consequences. Trimethoprim/sulfadoxine is classified as more important to human medicine (Category 2) compared to florfenicol and tetracycline (both Category 3) as categorized

by Health Canada (2009). Based on the current results, metaphylaxis use decreased phenicol and trimethoprim resistance in *E. coli* and *M. haemolytica*, suggesting that fewer antimicrobials from these classes were used due to fewer treatment failures necessitating treatment with these antimicrobials, with beneficial implications in public health.

Dynamic modeling provides a unique opportunity to examine the various complex interactions in microbial population. Agent-based modelling addresses some of the challenges to the studying AMR recognized with traditional approaches. As ABMs represent each organism as an individual, these models are uniquely able to model interactions between individual organisms at various levels of aggregation (Rutter et al., 2017). Unlike many other types of dynamic models such as System Dynamics models, ABMs can readily accommodate the structure of the feedlot with calves organized into pens that may be at different risks of disease. Decades of computational advances have made ABM a feasible tool to study a variety of complex and dynamic systems. Without these capabilities, it would not be possible to accurately predict the implications for AMR in antimicrobials used to treat individual animals at different treatment failure rates.

Similar to other modelling approaches, ABMs have both advantages and limitations. These advantages include stochasticity, heterogeneity, adaptation, and spatial structure (Bonabeau, 2002). The particular advantages of ABM come from its flexibility, which is attributable to the programmable rules imposed on the agents, while the environment may be simplistic or complicated. When modeling AMR in feedlot cattle, the number of agents involved and their resulting interactions can be combinatorially large, making it impractical to manually characterize these dynamics. Combinatorial explosion makes it impractical to use traditional modelling paradigm based on systems of differential equations. Agent-based modelling overcomes this limitation by specifying interactions and their dependencies between agents using rules (Kaul and Ventikos, 2015). This ability enables ABMs to conduct more powerful and perspicuous modeling with greater applications for use. However, overly complicated ABMs are more difficult to analyze. As well, when the number of model parameters increase, the space of possible parameter settings can become prohibitively too large to be searched efficiently (Lee et al., 2015). These challenges can be minimized by appropriately representing behaviour mechanisms and obtaining data to calibrate those mechanisms and validate the model (Badha et al., 2018).

The use of ABMs to guide public health interventions for the prevention of AMR is becoming more common. However, their application in veterinary medicine research, and specifically in livestock settings, is still very limited (Ramsay et al., 2018). Previous research involving ABMs to investigate the spread of multi-drug resistant bacteria are predominantly associated with community or hospital settings (Kardaś-Słoma et al., 2011; Suthar et al., 2014; Almagor et al., 2018) or the effect of intervention strategies (D'Agata et al., 2007; Kardaś-Słoma et al., 2013). Therefore, to the best of our knowledge, the current study is the first to have utilized ABM as a tool to model AMR in feedlot cattle with parameters based on empirical data. Agent-based models can identify critical areas where data are missing and generate hypothesis. Future applications of ABM have the potential to support traditional observational and experimental approaches in a complementary way.

Bovine respiratory disease complex represents the major driver of AMU in feedlot cattle, and thus reducing BRD incidence would significantly decrease the amounts of antimicrobials administered for therapy. Numerous studies have demonstrated the efficacy of metaphylaxis using various antimicrobial agents in preventing morbidity and mortality related to BRD (Step et al., 2007; Johnson et al., 2008; Abell et al., 2017; O'Connor et al., 2019; Word et al., 2020). In terms of economic benefits, Dennis et al. (2020) reported that the metaphylactic use of "upper tier" antimicrobial agents such as macrolides allowed producers to realize a net revenue from $58 to $119 higher than if no metaphylaxis was performed. This net benefit ranged from only $14 to $42 when "lower tier" antimicrobials such as sulfonamides or phenicols were used. In the case where no antimicrobials are used for disease prevention and control, decreasing the feeder cattle price by 9%, or alternatively, increasing the slaughter cattle price by 6.3%, would be necessary to offset the net revenue losses for the feedlot operator (Lhermie et al., 2020).

There is scientific support for the use of metaphylaxis in high-risk, newly weaned feedlot calves. High-risk calves that were administered tulathromycin metaphylaxis had shown reduction in BRD and improved performance (Baptiste and Kyvsgaard, 2017; Booker et al., 2007; Dennis et al., 2020; Munoz et al., 2020; Szasz et al., 2019; Tennant et al., 2014; Word et al., 2020). In terms of resistance prevalence, Doster et al. (2018) examined the effects of tulathromycin on the fecal resistome of feedlot cattle. The authors observed no significant effects on the resistome between treated and untreated groups. A cohort study by Lowrance et al. (2007) found that parenteral administration of CCFA to feedlot steers was associated with transient increase of

resistant E. coli, which returned to the pre-treatment levels 13 – 15 days following its administration. In the current study, the absence of metaphylactic AMU in high-risk calves arriving at a feedlot suggests a greater disease incidence and subsequently higher use of MIAs for therapeutic treatment. Thus, metaphylactic use of tulathromycin or oxytetracycline reduced resistance prevalence to antimicrobials used to therapeutically treat BRD. Therefore, metaphylaxis is warranted in order to reduce disease incidence throughout the feeding period. However, an increase in resistance prevalence was observed within the same drug class as the antimicrobial administered for metaphylaxis, antimicrobials that have lower importance to human medicine may be more appropriate for metaphylaxis in order to minimize the potential public health impact. Future research evaluating resistance prevalence for all classes of MIAs in a feedlot setting is warranted.

In western Canada, cattle at high-risk for developing BRD will most often be administered tulathromycin at the time of arrival to a feedlot (Brault et al., 2019a). Low risk cattle more likely to receive a long-acting oxytetracycline (Brault et al., 2019a). The antimicrobials from the macrolide class are also used extensively in the treatment of BRD for feedlot cattle (Brault et al., 2019a; Holman et al., 2019). As tulathromycin is an MIA, its metaphylactic use should be limited as increased AMR to macrolides has been reported in feedlot cattle raised with antimicrobials compared to those raised without AMU (Stanford et al., 2020). To avoid resistance selection of macrolides that could also be used to treat BRD, increased use of alternative antimicrobials such as oxytetracycline present a potential option for reducing AMR when used for metaphylaxis, and should be explored further in future studies.

In the current study, each experimental scenario was run for 10,000 iterations with 5 replications for each iteration. The large number of simulation runs was conducted in order to obtain a collection of simulation outputs with predicted resistance prevalence at various timepoints through the feeding period. Thus, 50,000 data points were collected for each of the experimental scenarios. A potential issue with this approach is applying small-sample statistical inference to large sample sizes since even minuscule effects can become statistically significant. Significant differences in resistance prevalence were observed between metaphylaxis protocols for TMS in *E. coli* on day 20, for TMS in *M. haemolytica* on day 20, and for TMS in *M. haemolytica* on day 225, although each instance was associated with values that differed very little in magnitude. Hence, the differences may not be clinically relevant despite being

statistically significant. A large number of observations are better than a smaller dataset as they provide greater statistical power and produce more precise estimates. However, the increased power leads to a possible drawbacks because these studies can detect smaller and more subtle effects. Thus, relying on p-value alone can lead to claims of support for hypotheses of little or no practical significance (Lin et al., 2013).

Agent-based models require simplification and assumptions given their complex nature. These models should be constructed as simple as possible, while being as complicated as necessary in order to address the predefined research questions. Therefore, some aspects relating to AMR in feedlot cattle were simplified or omitted from model completely given computational limitations or a lack of available data from literature. Resistance prevalence estimates emerging from the model are based on selection from AMU alone. Bidirectional exchange of antimicrobial resistant microorganisms can occur indirectly via the immediate environment and directly through contact with other animals in close proximity (Stevenson et al, 2003; Snyder et al., 2019; Zaheer et al., 2019). Confined feeding practices for large groups of animals, can increase opportunities for rapid spread (Bengtsson and Greko, 2014). Stevenson et al. (2003) reported that high levels of rifampicin-resistant strain of *E. coli* are readily transferred among cattle within the same pens, while Snyder et al. (2019) suggested the high level of clonal spread of *M. haemolytica* in individual calves may be due to contagious spread between calves. Additional research is needed to determine the impact of the environment in the spread of both AMR *E. coli* and *M. haemolytica* in the feedlot setting. The current version of the model represents a most extreme case scenario for the evaluation of metaphylaxis on arrival where all AMR is the result of selection due to drug use without the inclusion of additional environmental transmission and contagious dissemination pathways.

Another potentially important limitation of the current model was the incremental developmental decision not to account for MDR at this time. Multidrug resistance can be the result of co-selection processes, even in the absence of antimicrobial selection pressure (Sundqvist et al., 2010). Since resistance genes for many antimicrobial agents are placed on conjugative plasmids and ICEs, exposure to different antimicrobials can introduce selective pressures that maintain resistance for unrelated drugs.

The emergence of plasmids among *E. coli* is of particular concern. The presence of transferable plasmids encoding MDR and their dissemination among different enterobacterial

species may represent an increased public health risk as a source for spread of MDR determinants (Geser et al., 2012; Szmolka and Nagy, 2013). In recent years, MDR has been repeatedly documented in *M. haemolytica* isolates obtained from cattle (Lubbers and Hanzlicek, 2013; DeDonder and Apley, 2015; Woolums et al., 2018). Many of the AMR genes in these isolates are associated with ICEs, and these ICEs can harbor extensive MDR cassettes (Klima et al., 2014b, 2020; Eidam et al., 2015; Cameron et al., 2019; Stanford et al., 2020).

Multidrug-resistant elements can establish and persist in microorganism populations as the result of the use of a single antimicrobial that co-selects for the entire element (Klima et al., 2014b). These additional factors influencing AMR in feedlot cattle were not considered in the current model but could be included in future model iterations. Further examination is warranted to determine if most AMR observed is due to plasmids and ICEs that are transferred and then modified through gene loss, or uptake dependent upon selective pressures.

Historically, MDR *M. haemolytica* has not been highly prevalent in feedlot cattle (Noyes et al., 2015). However, more recent research in Canada (Anholt et al., 2017; Timsit et al., 2017) and United States (Lubbers and Hanzlicek, 2013; Snyder et al., 2017, 2019; Woolums et al., 2018) suggests that it is not uncommon to see strains of *M. haemolytica* resistant to more than one class of antimicrobial agents in feedlot cattle. The potential for MDR is high for *M. haemolytica* shed by high-risk animals that have been treated previously for BRD (Woolums et al., 2018). Lubbers and Hanzlicek (2013) reported *M. haemolytica* isolates that were resistant to five or more antimicrobials increase from approximately 5% in 2009 to approximately 35% in 2011. Further, Klima et al. (2014b) observed that diseased cattle were more likely to possess MDR *M. haemolytica* (37% of the population) compared to healthy cattle (2% of the population), while approximately 30% of the isolates were resistant to more than seven antimicrobial classes. Finally, Klima et al. (2020) showed an increase in prevalence of MDR *M. haemolytica* isolates from post-mortem lung tissues between 2011 to 2017, with 31% of isolates exhibiting resistance to more than seven antimicrobials. Therefore, delivery of diseased animals into the feedlot may increase the risk of introducing and propagating MDR in the feedlot. However, our model assumes animals arriving at the feedlot were healthy.

While fewer studies are available evaluating MDR in *E. coli*, observed prevalence is apparent. Benedict et al. (2015) analyzed isolates using two different techniques to determine resistance. Both methods were consistent in their findings; over 75% of isolates were susceptible

to all antimicrobials in calves sampled on arrival to a feedlot, although this number dropped 26.3% or less when sampled again at placement of hormone implants. Overall, more than 98% of samples showed resistance to three or fewer antimicrobials when sampled on arrival, and this value decreased to less than 93% on second sampling.

It should be noted that previous research investigating MDR in feedlot cattle have utilized samples from animals that were diagnosed or had died from BRD, or alternatively, from live animals regardless of health status. Studies that examined only morbidity and animals that succumbed to BRD may have biased results due to a higher potential for treatment failure (Lubbers and Hanzlicek, 2013; Anholt et al., 2017; Klima et al., 2014b, 2020). Fewer studies have been conducted in feedlot settings where both healthy and diseased animals were surveyed (Snyder et al., 2017, 2019; Timsit et al., 2017; Woolums et al., 2018), which are more relevant for comparisons to the current study.

As there appears to be a trend for increased AMR prevalence and MDR among isolates from feedlot cattle, exclusion of MDR limits our current findings. Studies have shown that resistance genes for macrolides and tetracyclines can be groups together (Michael et. al., 2012; Klima et al., 2014b). These studies suggested that resistance genes are part of ICE that can travel across genus boundaries. Thus, the likelihood that co-selection of clinically important AMR genes is a significant event which may contribute to the emergence of AMR microorganisms. Multidrug resistance may have far-reaching implications and that further research should be undertaken to investigate.

Finally, the requirement to identify current and evidence-based values for model parameters is a challenge. In the case of computational models, parameter values are typically determined using literature searches and consultations with subject matter experts, recognizing that relevant information is often not available. To validate such a model, computer output must be accurately fit to field data by choosing parameter values that yield the best match between simulation output and real-life observations. Model calibrations have proven to be difficult in practice, particularly due to their non-linear and resource-intensive nature. Davis-Unger et al. (2019) evaluated data from 28 feedlots over 10 years which reported 6.4%, 3.5%, and 0.8% cumulative incidence for BRD, footrot, and arthritis, respectively. However, metaphylaxis protocols were not known for that data set, and an epidemiological curve for treatments over the entire feeding period was not provided. Therefore, values from a five-year retrospective study

provided by an industry expert were used for the current model (N. Erickson, personal communication, June 8, 2016); cumulative incidences of BRD, footrot, arthritis were estimated to be 26%, 6%, and 1.1%, respectively. A plausible explanation for differences between the values used in this study compared to Davis-Unger et al. (2019) is variation of study populations. The data set analyzed by Davis-Unger et al. (2019) consisted of high and low-risk cattle, whereas the five-year retrospective study focused only on high-risk cattle. Additionally, comparative risk ratios obtained from O'Connor et al. (2019) were used to adjust for variation in the efficacy of metaphylactic treatments. This adjustment accounted for different BRD incidence rates based on the respective metaphylaxis treatment. However, the model does not address the effect of different metaphylaxis on the timing of BRD occurrence.

The time required to rear food-producing animals to a finished market weight differs between species given inherent differences between their respective production systems. In comparison to poultry and swine production, the feeding period for beef cattle is considerably longer. Thus, any AMR associated with metaphylactic antimicrobials administered to newly arrived cattle at high-risk of BRD have a longer period to wane before the animals are marketed, unless AMU occurs throughout the feeding period via parenteral or in-feed routes. Since metaphylaxis was shown to reduce therapeutic AMU later in the feeding period, the overall effect of parenteral AMU on animals at the time of slaughter is less due to more time available for waning.

The extended feeding period in feedlot cattle provides a plausible explanation for the decrease in AMR noted later in the feeding period (i.e., DOF 225). In the absence of selective pressure from AMU, there is evidence that lower acquisition of AMR is associated with a decrease in the fitness of the organisms (Andersson and Hughes, 2010; MacLean and Vogwill, 2015; Hernando-Amado et al., 2017). Furthermore, plasmids generally impose a fitness cost on the organisms for maintaining resistance (Starikova et al., 2013). While under selective pressure, plasmid-borne traits confer a benefit to their hosts, although this becomes detrimental once the pressure is removed (MacLean and Vogwill, 2015). Thus, it is expected that organisms lacking the resistance gene may be outcompeted due to the fitness cost of plasmid carriage.

For the current study, in-feed AMU for prevention of histophilosis and liver abscesses was considered in each scenario and resulted in a baseline selective pressure for AMR, regardless of the injectable metaphylaxis option. The experiments did not, however, evaluate the

specific additive effect of in-feed AMU since the project objectives were to only assess the influence of different injectable metaphylaxis options. All AMU has the potential to exert selective pressures, irrespective of their administration route. Research by Platt et al. (2008) showed that in-feed chlortetracycline was associated with a transiently increased proportion of resistant fecal *E. coli* and *Enterococcus* isolates for feedlot cattle. A study by Miller et al. (2018) investigated the effect of single 5-day in-feed administration of prophylactic chlortetracycline on the occurrence of resistant E. coli in feces. The authors found a temporary increase in tetracycline resistant *E. coli* population following chlortetracycline in-feed administration, although there was no difference between the treated and control groups. Similarly, Kanwar et al. (2013) reported that oral administration of chlortetracycline in feed following treatment with parenteral CCFA in feedlot cattle increased ceftiofur-resistant bacteria found in feces. It was suggested that a combination of chlortetracycline and CCFA may increase the risk of isolating AMR bacteria. Based on these findings, future research is warranted to examine the additive effect of injectable metaphylaxis and in-feed prophylaxis on resistance prevalence, particularly when considering the potential for MDR.

The present study illustrated that metaphylactic AMU restrictions may not result in a simple subsequent reduction in AMR to all antimicrobials of importance to human health. Metaphylaxis remains an effective tool to reduce morbidity and mortality related to BRD while potentially resulting in reductions in the use of some MIAs for BRD treatment. Early preventative treatment may reduce the overall number of sick animals while decreasing the amount of antimicrobials required for therapy later on in the feeding period.

2.6 Conclusion

In this study, we examined the potential impact of different options for injectable metaphylactic use on the prevalence of AMR in feedlot cattle in a typical western Canadian feedlot using an ABM. Current findings suggest that the absence of metaphylactic AMU in high-risk calves arriving at a feedlot could result in subsequently higher use of some MIAs for therapeutic treatment as a result of greater BRD incidence. Thus, metaphylactic AMU restrictions may not be a simple solution for reducing all types of AMR. Antimicrobial agents that have lower importance to human medicine should be selected where possible to reduce any potential public health impact. Metaphylaxis remains an effective way to reduce BRD for high-

risk feedlot cattle and subsequently therapeutic AMU for BRD treatment. Future researchers should consider investigating the impact of environmental transmission from either a reservoir of resistant bacteria or via animal-to-animal contact as well as MDR elements on AMR and how these findings might impact recommendations for antimicrobial stewardship. Regardless, in this study an ABM was developed that successfully illustrated the associations between injectable metaphylactic use and the prevalence of AMR in feedlot cattle. Our results demonstrated ABMs as a valuable tool to generate a theory and testing hypotheses leveraging available empirical data to address a complex systems problem.

CHAPTER 3
AN AGENT-BASED MODEL OF ANTIMICROBIAL RESISTANCE IN A WESTERN CANADIAN FEEDLOT EXAMINING THE ROLE OF TRANSMISSION AMONG ANIMALS AND THROUGH THE ENVIRONMENT

Michelle Thompson, Nathan Erickson, Sheryl Gow, Nathaniel Osgood, Cheryl Waldner

There is a critically important knowledge gap in the relative importance of other factors beyond AMU that can contribute to the development of AMR in feedlot cattle. Transmission of resistant organisms between animals and through the environment has not been extensively researched. A better understanding of potential routes for transmission of resistant organisms is required so that modifiable causal factors can be identified in order to reduce or prevent further spread of resistance. An ABM that included the transmission of resistance determinants through animal-to-animal contact and contact with manure was developed to simulate the selection of resistant *M. haemolytica* and *E. coli* following AMU. The study revealed that both drug use and contagious transmission can provide comparable explanations for changes in AMR during the feeding period for feedlot cattle. Therefore, contagious spread of AMR, either directly from animal to animal or through the environment, must be considered when evaluating the implications of policy changes to AMU and expected benefits to antimicrobial stewardship efforts.

Author contributions: Michelle Thompson was responsible for model design and implementation, data analysis, and manuscript preparation. Waldner, Erickson, Gow, and Osgood were responsible for study design and manuscript review.

3.1 Abstract

Antimicrobial resistance (AMR) represents a significant challenge for the effective prevention and treatment of bacterial infections in humans and animals (WHO, 2017). Antimicrobial use (AMU) in livestock production is often scrutinized due to the volume (kgs) of antimicrobials used and the perception that this AMU is contributing to AMR development. However, transmission of resistant organisms through the environment and between animals is a critically important knowledge gap. An agent-based stochastic model (ABM) that allowed for the transmission of resistance determinants through animal-to-animal contact and contact with manure was developed to simulate the selection of resistant *M. haemolytica* and *E. coli* following AMU. The objective of this study was to explore the role of transmission in the spread of AMR amongst feedlot cattle. The model illustrated that both drug use and contagious transmission can provide comparable explanations for changes in AMR during the feeding period. Scenarios including environmental transmission only produced greater variability in resistance prevalence and were potentially a better fit to observed data in comparison to scenarios involving drug use only and the combination of drug use and transmission. The ABM designed in this study successfully replicated previously observed resistance prevalence for *M. haemolytica* and *E. coli* in a typical western Canadian feedlot setting assuming resistance was due to AMU, transmission of AMR, and a combination of both. These findings indicate that the transmission of AMR either directly from through animal-to-animal contact or through the environment must be considered when evaluating the implications of policy changes to AMU and the expected benefits to antimicrobial stewardship efforts.

3.2 Introduction

Antimicrobial use in food producing animal species, including beef cattle, is facing increased public scrutiny due to concerns related to the potential transfer of resistant pathogens to humans. Antimicrobial resistance (AMR) is a deepening challenge in human and veterinary medicine worldwide (Prestinaci et al., 2015; Dadgostar, 2019). The World Health Organization (WHO) (2014) reported that the world is on the cusp of a "post-antibiotic era" where untreatable infections would emerge on a massive scale. Although AMR is an inevitable consequence of the evolutionary adaptation of microorganisms, use and misuse of antimicrobial agents have driven the increasingly rapid emergence of resistance in a range of both pathogenic and commensal organisms (Levy, 1982; Davies and Davies, 2010).

Understanding transmission of resistant pathogens among animals is central to managing AMR in feedlot cattle. However, the relative importance of factors beyond AMU in the dissemination of AMR amongst feedlot cattle is unknown, due in part to the complex system of risk factors influencing pathogen exposure and disease risks. A better understanding of potential routes for transmission of resistant pathogens is required to identify modifiable causal factors that could reduce or prevent further spread of resistance. Typically, the main goal of antimicrobial stewardship programmes is to regulate and promote prudent use of antimicrobial agents to lessen the risk of AMR. However, without inclusion or consideration of all the pathways for AMR transmission, mitigation plans are incomplete and may not improve existing and future antimicrobial susceptibility.

The contagious spread of bovine respiratory disease (BRD) pathogens, particularly *Mannheimia haemolytica* (*M. haemolytica*), has been documented in feedlot settings (Shane et al., 2018; Strobel et al., 2018; Synder et al., 2019). Shane et al. (2018) investigated animal-to-animal contact patterns within the first 28 days of arrival at the feedlot, and reported an increased risk of BRD when there was increased contact between calves shedding BRD pathogens. The frequency with which animals contacted potentially contaminated environmental sources has also found to be important for determining BRD spread (Shane et al., 2018; Strobel et al., 2018). Calves separated by time of arrival and by physical barriers, contracted the same *M. haemolytica* strains as calves already present and infected, again showing the importance of environmental sources in the spread of BRD (Synder et al., 2019). The Synder et al. (2019) study suggests that

short-term holding pens could serve as a point source of infection, adding to animal-to-animal contact and potential airborne transmission of BRD.

Similarly for AMR in fecal organisms there is evidence that AMU alone does not explain the patterns of observed resistance in feedlot pens (Graham et al. 2019). Peak et al. (2006) suggested that transmission of antimicrobial resistant fecal organisms among animals was a plausible explanation for the similarities seen in AMR between environmental reservoirs, including manure, associated with groups of cattle that had and had not been administered antimicrobials. Earlier Stevenson et al. (2003) had also documented the transmission of AMR *Escherichia coli* (*E. coli*) within feedlot pens at a research facility.

Agent-based models (ABM) are stochastic models used to describe populations of interacting agents using simple rules that dictate their behaviors and thus simulate the heterogeneity within and among populations. The aggregate behavior of the simulated system arises from the joint effects of the relatively simple actions of the simulated individual members of the population or agents. Agent-based models are a natural fit for modelling AMR in a feedlot setting due to the dynamic interactions between situated individuals, and individual interactions with local environments. Furthermore, ABMs are capable of handling combinatorial complexities by avoiding formulating differential equations for individual agents (Kaul and Ventikos, 2015). Additionally, ABM provide an alternative or supplemental option to extensive field intervention studies to explore hypothetical system-wide consequences of policy and management changes. The flexibility to examine the unique characteristics of individual agents, levels of aggregation of those agents, interventions, and their interactions make ABM an ideal tool for analyzing complex biological phenomena, such as AMR epidemiology, without making prior assumption as to how the system will be affected (D'Agata et al., 2007).

The objectives of this study were to develop and use an ABM to examine the selection and dissemination of AMR in a typical Canadian feedlot. The study compared the potential for oxytetracycline use alone to transmission (i.e., animal-to-animal, environmental) and a combination of AMU and transmission in explaining observed oxytetracycline resistance in *M. hemolytica* and *E. coli* in feedlot cattle. For the purpose of this modeling experiment, transmission of AMR was assumed to be primarily based on direct animal to animal contact for *M. haemolytica* and as a result of fecal contamination of the environment for *E. coli*.

3.3 Materials and Methods

A continuous-time, stochastic agent-based simulation model was constructed with AnyLogic® 8.7.4 simulation software (XJ Technologies, Saint-Petersburg, Russia) using Java-based code. The model simulates typical western Canadian feedlot management and examines the relative contributions of direct transmission among calves and environment contamination relative to selection for AMR by AMU alone. There are several standardized model documentations, such as the Overview, Design concepts, and Detail (ODD), ODD + Decision (ODD + D), and ODD + D + Data (ODD + 2D). The ODD + D and ODD + 2D had been proposed as the extensions of the ODD protocol as a new standard format for describing ABMs (Laatabi et al., 2018; Müller et al., 2013). In the current study, the ODD protocol was used to describe the model. The ODD protocol's Overview section first introduced the model's agents and their basic interactions, the Design concepts section describes the general principles of the model's design, while the Details section describes the rules that govern the model's operation (Grimm et al., 2010).

3.3.1 Overview

Purpose

This study intended to address whether including the potential for AMR transmission better explains the observed prevalence of AMR compared to a scenario where all selection for AMR was the result of AMU. In this model, we targeted oxytetracycline resistance because the prevalence of resistance to tetracyclines was higher and exhibited greater variability than for the other antimicrobials in the available empirical data (Benedict et al., 2015; Noyes et al., 2015). Further, oxytetracyclines have been extensively used in the therapy of human and animal bacterial infections as well as for metaphylaxis and prophylaxis in feedlot cattle (Brault et al., 2019a).

The objective was to compare the performance in explaining observed trends in AMR prevalence during the feeding period of: 1) a model allowing only for changes in AMR due to AMR transmission; 2) a model in which AMR was dependent only on selection due to AMU; and, 3) a final model which featured a combination of both transmission and AMU-associated selection. Transmission could be via fecal contamination for *E. coli* or through contact among animals in close proximity for *M. haemolytica*. *M. haemolytica* was considered as a surrogate for

respiratory pathogens and *E coli* as a surrogate for fecal commensal organisms. An ABM was developed to compare: 1) resistance to oxytetracycline as a result of selection based on injectable oxytetracycline use at arrival and in the feed; 2) resistance to oxytetracycline associated with transmission of AMR in respiratory pathogens present at arrival either from animal-to-animal contact, or in fecal commensals as a result of transmission of AMR through fecal contamination of the environment; or 3) both AMR selection based on oxytetracycline use and transmission of AMR.

Entities, state variables, and scales

The system was modeled as a collection of autonomous entities called agents. The model described four types of agents organized in a hierarchy: feedlot made up of pens, filled with cattle containing sentinel organisms that were either sensitive or resistant to a set of commonly used antimicrobials. Each agent was assigned internal states and behaviours encoded by rules which govern the transitions between states over time. Interactions between agents produced emergent, system-level dynamics by taking into account of the environment and reacting to changes within it.

The **feedlot agents** governed the processes of calf arrival, allocation of calves to pens and re-sorting of calves later in the feeding period. The feedlot consisted of individual pens, with each pen containing its own calves and environmental reservoirs.

The **pen agents** recorded the filling and emptying of the pen and governed pen-level prophylactic and metaphylactic AMU throughout the feeding period. Management protocols employed at the feedlot for disease control that included antimicrobials and potentially resulted in selective pressure for AMR included: injectable metaphylaxis with tulathromycin on arrival for BRD management, in-feed chlortetracycline prophylaxis for prevention of histophilosis and in-feed tylosin for prevention of liver abscesses. Specifically for this configuration, chlortetracycline was added to the feed on day 18 for a total of 10 days as a pulse (five days on, two days off and five days on). Thereafter, tylosin was introduced into the feed on day 30 and was continued until calves reached their target market weight. Once the average weight within a single pen reached 1400 pounds, calves were assumed to have met their target market weight and were ready for slaughter. Pens were then de-populated and pen summary data were exported.

The **environmental reservoir agents** represented the amount of contamination with fecal AMR sentinel bacteria present in the pen. This modeling construct included the potential for residual manure contaminated with AMR bacteria in the pen from previous groups of cattle, fresh manure contaminated with AMR bacteria deposited into the pen or on surfaces, and AMR bacteria in feces on the hides of the animals creating a risk through grooming behaviours. Further, each environmental reservoir agent represented resistance to one of the 11 antimicrobials specified in the model. The environmental reservoir was maintained through accumulation of fecal matter containing antimicrobial resistant organisms. Each animal in the pen excreted one unit of fecal matter per day, and that the feces contained organisms resistant to each of the drug classes to which the animal carried resistance. The likelihood of acquiring resistant *E. coli* through contact with fecal matter was in part determined by the current amount of these resistant organisms in the manure comprising the environmental reservoir. The accumulated manure in the pen decayed at a calibrated rate, thereby reducing the environmental reservoir of resistant organisms over time. No differentiation between fresh or decaying manure was made during the feeding period. Rather, the reservoir was calculated by applying a calibrated decaying coefficient to the previous days' carryover amount, and then adding the current days' accumulation to the overall total at the conclusion of each day. After calves were shipped for slaughter at the end of the feeding period, the pen was cleaned removing a specified proportion of the reservoir material but not disinfected. Any residual manure containing resistant organisms was carried over to the next feeding period and contributed to the environmental reservoir for the subsequent animals entering the pen.

The **animal agents** characterized the health and treatment status for calves as well as the isolation of chronically sick calves. Healthy calves could become sick and be diagnosed with either BRD, arthritis or foot rot at rates consistent with the baseline management protocol. Thereafter, the antimicrobial agent specified in the feedlot protocol would be administered as a therapeutic treatment and individuals would be returned to their respective pens. Animals treated for BRD more than three times were sent to the chronic sick pen and stayed there for the remainder of the feeding period. Sick calves treated towards the end of the finishing period were removed from their regular pens and shipped for slaughter at a later date as required to adhere to antimicrobial withdrawal times. The number of treatments per calf was tracked and reported.

AMR status for each population of sentinel organisms within each calf was governed by a state chart specific for each sentinel organism. Each **resistance agent** represented one of the 11 antimicrobials used in the model. Calves that just arrived at the feedlot might have already been colonized with resistant organisms based on the probability of resistance on arrival. Selection, transmission and waning of AMR for a given population of sentinel organisms was assumed to occur uniformly across each drug class based on rates calibrated by fitting the model to historical data (Benedict et al., 2015; Noyes et al., 2015).

Process overview and scheduling

The model described a typical western Canadian feedlot consisting of pens populated by high-risk calves placed in the fall. These high-risk calves would typically be recently weaned, lighter weight beef calves purchased from auction and could already have been in the early stage of disease or incubating the disease (Brault et al., 2019a); this scenario was assumed to represent the highest risk of AMR selection and dissemination. The model time unit was days and events occurred at either 1) an arbitrary point in time, driven by incidence rates, or as a consequence of other events (e.g., receipt of a "transmission" message sent by an agent); or 2) a fixed time, following the occurrence of another event (e.g., animals arriving at the feedlot, re-sorting animals after a certain number of days on feed (DOF), treatment after disease diagnosis).

Each model run simulated a cohort of cattle arriving at a 6,960-head feedlot over either a one or three-year period. The virtual feedlot represented by the model was of moderate size and contained 29 home pens (240 calves per pen) and one chronic sick pen. The pens were arranged in rows and filled successively one after another from left to right. A random number of pens at a time – between 1 and 4 – were filled at random intervals, increasing in frequency from the start of the fall run in October and peaking in November before slowing slightly until the feedlot reached full capacity in December. Individual pens housed exactly 240 fall-placed calves that were assumed for model parsimony to arrive at the virtual feedlot on the same day prior to being placed into the same pen. All calves were considered high-risk and received injectable metaphylaxis upon arrival, with prophylactic antimicrobials delivered to cattle via medicated feed.

Calves from pens that had been on feed for 80 to 100 days were periodically pooled together and re-sorted for the purpose of marketing uniform groups of cattle. Specifically, calves

that have been pooled together from different pens are ranked based on body weight from lightest to heaviest. Groups of 240 calves would then be consecutively distributed into their new pen according to body weight (i.e., the lightest 240 calves would enter one pen, then next 240 calves ranked by weight would enter a separate pen, and so forth until the entire group was redistributed). Once the entire pen reached an average weight of approximately 1400 pounds, all cattle were shipped for slaughter and processing.

Cattle entered the feedlot in a healthy state; individuals were monitored daily throughout the feeding period to identify presence of disease. If a calf became sick, therapeutic treatment was administered within 12 hours, according to manufacturers' guidelines. For diseases such as arthritis and foot rot, therapeutic treatment was assumed to be effective, and the calf returned to a healthy state after the administered antimicrobial's duration of therapy had lapsed. For BRD, treatment was assumed to be ineffective in a proportion of cases due to the extensive disease progression before diagnosis, medication errors, or altered pharmacokinetics of antimicrobials due to pathophysiologic changes in the host animal (Booker and Lubbers, 2020). However, the probability of BRD treatment failure also increased dynamically as an emergent outcome of the model and represents a direct consequence of the average pen prevalence of *M. haemolytica* resistant to the drug class used for treatment.

Selection of resistant *M. haemolytica* and generic fecal *E. coli* were monitored throughout each feeding period, allowing for evaluation of both direct respiratory transmission among animals and primarily indirect transmission through fecal contamination of the environment in the spread of AMR.

3.3.2 Design concepts

Basic principle. The use of antimicrobial agents provides a selection pressure that acts on a population, selecting for resistance genes that favor the survival of resistant microorganisms. AMR can also be disseminated through the transfer of resistance genes and/or microorganisms directly among animals and indirectly through the environment.

Emergence. Antimicrobial resistance development that could lead to a treatment failure, which in turn would impact subsequent treatment rates and the antimicrobial selected to treat BRD all emerged directly from the model. Selection and waning of AMR in feedlot calves occurs during the feeding period in response to AMU on arrival for metaphylaxis, in feed and for treatment of

disease. Metaphylaxis also influenced the frequency and types of antimicrobials being used for treatment of BRD leading to dynamic emergent AMR patterns for the drugs used for treatment. This relationship is made more complex by the potential for treatment failure, in some cases resulting from AMR, and the need for further AMU to increase dynamically with emerging AMR.

All calves in the same pen were assumed to have an equal probability of acquiring resistance via contact. Calves could also potentially spread resistant *M. haemolytica* to other "connected" calves in their social network (detail discussed to follow).

In the feedlot model, transmission of fecal AMR (e.g., *E. coli*) through the external environment depends upon the level of environmental contamination. Furthermore, each pen was considered to have its own microenvironment, with a resistance reservoir corresponding to each individual antimicrobial that accumulated as resident calves with resistant *E. coli* defecated in the pen. Acquisition of generic *E. coli*-carrying AMR genes by animal hosts can occur through contact with fecal matter in the pen. As the resistance reservoir grew, the likelihood of acquiring resistance also increased.

Stochasticity. Once animals were randomly placed in the model environment, they were connected to other animal agents within the same pen in a contact network. Calves could acquire resistance through random contact with other calves in the same pen or through stochastic fecal exposure from the environment. Incidence rates of both arthritis and foot rot were also randomly selected from predefined ranges derived from observations by industry expert (N. Erickson, personal communication, June 8, 2016). Finally, selection and waning of AMR was based on a rate which exhibited an exponentially distributed timeout (i.e., rate = 0.5/hour, timeout will have a mean value of 1/0.5 = 2 hours).

Collective. The model was organized as a hierarchy. The feedlot contained the aggregation of individual pens, each pen with a specific group of calves assigned at the time of arrival, and each calf with its own sentinel bacteria that were either sensitive or resistant to a set of commonly used antimicrobials. Further, a transmission network was established for each calf based on user specification. By default, a local transmission network was implemented where calves within the same pen were "connected" to each other.

Observation. The mean resistance prevalence of *E. coli* and *M. haemolytica* for all pens in the feedlot was calculated and updated graphically on a daily basis. Only drug classes for which

resistance could be meaningfully interpreted were reported. For *E. coli*, these drug classes were cephalosporin, fluroquinolone, trimethoprim, sulfonamide, phenicol and tetracycline. The same drug classes were reported for *M. haemolytica* in addition to macrolides. Intrinsic resistance was defined as innately possessing resistance mechanisms against the respective drug class. *E. coli* contains mechanisms that confer innate resistance to macrolides and penicillins, and *M. haemolytica* possesses mechanisms that confer resistance to penicillins (Plumb, 2018; Giguère et al., 2013), excluding these drug classes from reporting. Occurrence of respiratory disease, arthritis, and foot rot treatments as well as the BRD chronic case incidence rate were also reported. The calculated resistance prevalence time series were then exported to an Excel spreadsheet for subsequent analysis.

3.3.3 Details

Initialization

The model was initialised as a newly stocked feedlot without an existing environmental reservoir at the beginning of either a one- or three-year cycle. Each production cycle started on the 1st of October. A three-year cycle was used to examine environmental transmission of AMR *E. coli* to allow for build up of an environmental reservoir. The first two production cycles were considered a warm up period or a burn-in time to allow the environmental reservoir to achieve conditions that more closely resembled normal running conditions of a typical feedlot. Only data from the third production cycle was used for analysis. A one year cycle was used for all other scenarios.

Input

Parameter inputs were obtained from the peer-reviewed literature and expert opinion from team members (Table 3.1). Herd management practices and antimicrobial treatments used in the baseline scenario are provided in Table 3.1. A five-year retrospective feedlot study was referenced for first treatment incidences in fall calves receiving tulathromycin or tildipirosin on arrival (N. Erickson, personal communication, June 8, 2016). In this study, first treatment incidences averaged 0.06% per day at day 5 on feed, peaking at 1.14% by day 25 and stabilizing to between 0.05 and 0.10% from days 60 to 100.

Table 3.1: Values used to parameterize the feedlot and animal management in the baseline scenario of the ABM.

Parameter	Value
Number of animals per pen	240
Allow metaphylaxis	True
Allow in-feed prophylaxis	True
DOF to begin first round of in-feed prophylaxis (chlortetracycline)	18th day on feed
Duration of in-feed prophylactic use	5 days
DOF to begin second round of in-feed prophylaxis (chlortetracycline)	25th day on feed
DOF to begin tylosin in-feed prophylaxis	30th day on feed
Product used for injectable metaphylaxis	Tulathromycin
BRD first treatment incidences	Frequency distribution of first treatment for BRD specific to DOF from 5-year retrospective analysis (unpublished data); Cumulative incidence: 26%
Baseline probability of failure for first BRD treatment	Selected from uniformly distributed double value between 10 and 25% or relative frequency of AMR if greater than baseline value of treatment failure for first BRD treatment option
Baseline probability of failure for second BRD treatment	50% or relative frequency of AMR if greater than baseline value of treatment failure for second BRD treatment option
Baseline probability of failure for third BRD treatment and movement to the chronic pen	Relative frequency of AMR
Arthritis incidence	Selected from a uniform distribution between 0.6 and 1.6% per week from days 30 to 100 on feed; Cumulative incidence: 1.1%
Foot rot incidence	Selected from a uniform distribution between 2 and 12% per week over 245 days during the feeding period; Cumulative incidence: 6.0%

Reference: N. Erickson, personal communication, June 24, 2016

The antimicrobial agents used for therapeutic disease treatment and a list of therapeutic treatments used for all scenarios in the model are listed in Table 3.2. A variation option allows the user to select the default treatment option, or alternatively lets the model randomly select a treatment from a list of commonly used antimicrobial agents (N. Erickson, personal communication, June 8, 2016). For example, when calves were not successfully treated with florfenicol as the first BRD therapeutic treatment, one of two additional antimicrobial agents

(ceftiofur and enrofloxacin) were randomly selected as the second treatment options. In cases where an animal required a third treatment for BRD, trimethoprim sulfadoxine (TMS) was listed as the therapeutic choice. Calves that did not respond after a third treatment were sent to the chronic pen for the duration of the feeding period.

Tulathromycin, florfenicol and oxytetracycline were listed as common therapeutic treatments for arthritis used in western Canadian feedlots. For each simulation run, one of these three antimicrobials were selected at random and utilized throughout the feeding period to treat calves affected by arthritis. One of ceftiofur or penicillin were randomly selected at start-up for use as therapeutic treatments for foot rot.

Table 3.2: Treatment protocols for BRD, Arthritis, and Foot Rot.

	Notes
BRD	
1st treatment	Florfenicol.
2nd treatment	Ceftiofur or enrofloxacin (randomly chosen at the feedlot-level at start up).
3rd treatment	TMS
Arthritis	Treated once using tulathromycin, florfenicol, or oxytetracycline (randomly chosen with equal probability at the feedlot-level at start up).
Foot rot	Treated once using ceftiofur or penicillin (randomly chosen with equal probability at the feedlot-level at start up).

Reference: N. Erickson, personal communication, June 24, 2016

Eleven antimicrobials were considered in the present version of the model and were further subdivided into classes (Table 3.3). Certain antimicrobials (e.g., TMS) belong to multiple drug classes due to their complex compositions and therefore these antimicrobials were included in each relevant drug class.

Antimicrobial resistance would begin to wane at rates specific for each drug class and type of bacteria after completing therapeutic antimicrobial treatment, metaphylaxis or prophylaxis as soon as the estimated duration of selective pressure for the administered drug had lapsed. The time required for a drug to be eliminated from the body once drug administration ceased was determined using elimination half-lives. Most drugs are eliminated by 3 to 5 half-lives (Merck Manual, 2015). Since values were not available for the duration that selective pressure was being exerted on microbial communities, three half-lives were chosen as a

conservative measure of effective selective pressure duration. These values can be modified to account for alternative hypotheses if more accurate data becomes available.

Table 3.3: Antimicrobials included within the model.

Category/Class	Antimicrobial	Withdrawal Time (days)	Estimated Effective Duration of Selective Pressure (days)*
Category I[1]			
Cephalosporins (3rd & 4th generation)[1]	Ceftiofur crystalline free acid	13	7.8
	Ceftiofur hydrocholoride	3	4.3
Fluoroquinolones	Enrofloxacin	36	0.8
Category II			
Macrolides[1,3]	Tulathromycin	44	8.3
	Tylosin	0	0.1
Trimethoprim[2,3]	Trimethoprim/Sulfonamide	10	1.4
Penicillins[1,5]	Penicillin	5	2.3
Category III			
Phenicols[1]	Florfenicol	55	6.7
	Florfenicol/Flunixin	60	6.7
Tetracyclines[1,4,6]	Oxytetracycline	48	2.7
	Chlortetracycline	5	2.0
Sulfonamides[1,3]	Trimethoprim/Sulfonamide	10	1.4

[1]Bayer Corporation, 1991
[2]Riviere et al., 2003
[3]Plumb, 2018
[4]Toutain and Raynaud, 1983
[5]Papich et al., 1993
[6]Reinbold et al., 2010
*Effective duration of selective pressure was estimated using the half-life of each antimicrobial × 3.

On-arrival resistance prevalence to each drug class for *M. haemolytica* (Noyes et al., 2015) and *E. coli* (Benedict et al., 2015) were obtained from respiratory and fecal samples collected between 2007 and 2010 as part of a large surveillance project conducted in western Canada. The study authors supplied additional data to allow calibration of product and organism-specific selection and waning rates to best fit reported resistance prevalence at day 45, 90, 135, 180 and 225 from 2007-2008 (P. Morley, personal communication, 2018). Drug products within the same antimicrobial class were assumed to share the same susceptibility to resistance

mechanisms and to have the same selection and waning rates. This simplifying assumption can be modified as more specific data become available.

Submodels

There are three submodels operating as a part of the main model, each describing different processes that run simultaneously as the model executes. The three submodels are (1) cattle agent disease cycle; (2) selection and waning of AMR in sentinel organism agents; and (3) transmission of AMR via direct contact between animals in close proximity and indirect contact via environmental components such as manure.

In the model, the disease process in the *cattle agent* is modelled using a single state chart which regulated the health status of the calf. Healthy calves could become sick and be diagnosed with either BRD, arthritis or foot rot as mutually exclusive states within the same state chart. In the case of animals diagnosed with BRD for the first time, treatment failure was determined using either the pen level resistance prevalence or a predefined incidence rate (10 – 25%), whichever had a higher value. When animals were diagnosed a second time, the same factors were considered, although the rate of treatment failure increased to 50%. For animals with a third BRD diagnosis, pen level resistance prevalence alone was used to determine treatment failure. If the calf received three BRD treatments without success, then the animal was moved to the chronic pen. Calves diagnosed with arthritis or foot rot were administered one treatment which was assumed to be successful, and calves were returned to healthy status. These treated calves were at risk of subsequently acquiring a second, independent infection following a successful treatment.

Selection and waning of AMR was modelled with explicitly parallel state charts for each type of antimicrobial, which governed how selection and waning of AMR occurred within each bacterial population within each calf (Figure 3.1). Upon arriving at the feedlot, calves might have already been colonized with resistant organisms in accordance with the probability of resistance on arrival. When an antimicrobial was administered during the feeding period at the feedlot, messages were sent to the corresponding resistance state charts. A susceptible bacterial population within a calf could become resistant as prescribed by the rate of AMR selection with respect to specific class of antimicrobial. Within that bacterial population, AMR for that particular class of antimicrobial was assumed to wane once selective pressure from AMU stopped. The estimated duration of selective pressure was assumed to be based on the half-life of

the drug (Table 3.3). Selection and waning of AMR was assumed to occur uniformly across the drug class, such that once an antimicrobial was administered, resistance to other antimicrobials within the same drug class would likewise undergo selection and waning.

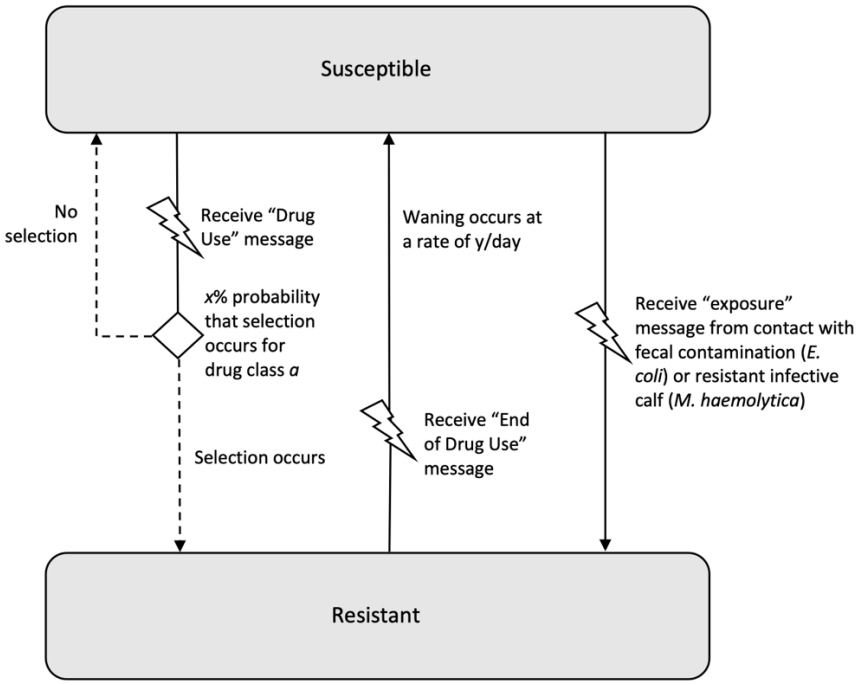

Figure 3.1: Process overview of AMR in the ABM**.** Diamonds represent chance outcomes; dotted lines represent options that could occur given a true or false outcome. Lightning bolts indicate the sending or receiving of messages to/from other agents (i.e., pen, animal, organism). The selection probabilities for resistance are different and specific to a particular drug class and type of sentinel organism (e.g. *M. haemolytica* or *E. coli*).

In addition to AMU, contagious spread of a resistant microorganism can occur through contact with other animals in their proximity. The transmission of resistant *M. haemolytica* between calves occurred through physical contact or proximity. Animals residing in the same pen were connected to each other and transmission of resistant organisms could occur among pen-mates. An individual animal was hypothesized to expose resistance to two animals in the same pen each hour, potentially exposing resistance to 48 other animals in the pen each day or potentially all animals in the pen in five days. Exposure messages did not necessarily lead to the receiving agent becoming resistant. A rate transition operationalized as a probability per day of an animal acquiring resistant *M. haemolytica* was based on a calibrated parameter.

The influence of the external environment contributing to AMR was also considered in the model. *Transmission of AMR through the external environment* depended upon the level of environmental contamination. Each pen was considered to have its own microenvironment with a resistance reservoir that accumulated as resident calves defecated in the pen. It was assumed that each calf excreted one unit of fecal matter per day, and that the feces contained organisms resistant to each of the drug classes to which the calf carried resistance. Acquisition by animal hosts of generic *E. coli* carrying AMR genes can occur through fecal ingestion as a result of contact with other animal hosts (e.g., grooming behaviour) and/or fecal matter in the pen (considered primary source in this scenario). The likelihood of acquiring resistance through fecal contamination was based on a calibrated parameter. This likelihood was then multiplied by the current accumulated level of resistant organisms in the environment (reservoir) to produce a probability per day of acquiring resistance through fecal contamination. All calves in the same pen were assumed to have an equal probability per day of acquiring resistance via contact with manure.

3.3.4 Model calibration and statistical analysis of outcomes

Calibration refers to the process of systemically estimating model parameters seeking to make emergent behaviour of that model reproduce the observed data (i.e., resistance prevalence) as closely as possible. Parameter calibration represents a global optimization problem, where the aim is to find the best possible parameter sets x from a set X according to a predefined set of criteria F = $\{f_1, f_2,\ldots, f_n\}$ (Auchincloss and Garcia, 2015). The X represents the search space, which contains all acceptable combinations of all model parameter values. The set of criteria is defined with mathematical functions, which are called objective functions. In the feedlot model, the objective function was based on the sum-squared difference between model resistance prevalence output and observed resistance prevalence data collected from literature (Benedict et al., 2015; Noyes et al., 2015; P. Morley, personal communication, 2018). Calibration works to discover target parameter values that minimize the dissimilarity between model output and observed data by minimizing the objective function and determining the least-squared error. Model calibration was completed using a stepwise approach and the objective function from each of the calibration steps was compared to measure the fit of the model results to the observed

data. All model calibration experiments were completed separately for *M. haemolytica* and for *E. coli*.

The first part of the calibration assumed that selection and waning of AMR derived from AMU only, and did not consider transmission between animals or from fecal matter. An automated AnyLogic® calibration experiment was completed to determine the selection and waning rates associated with AMU tetracycline use assuming that tetracycline use was the only factor influencing changes in AMR. The experiment automatically varied the selection and waning rates over continuous ranges to minimize the objective function quantifying the discrepancy between the model output and empirical AMR prevalence data (Benedict et al., 2015; Noyes et al., 2015). To achieve optimized parameter values for each calibration experiment, the model was run for a minimum of 10,000 and 2,000 iterations over a period of one year for *M. haemolytica* and *E. coli*, respectively. Each iteration was run for minimum of six replications (realizations), after which they would stop provided the estimated objective value fell within 95% confidence level around the mean of replication results (i.e., objective values) to a maximum level of 10 replications.

The second calibration phase disabled selection of AMR due to drug use and examined instead both direct transmission between animals for *M. haemolytica* and indirect transmission via fecal *E. coli* contamination. The environmental and contact transmission parameters were calibrated in a similar fashion as in the first calibration phase. Empirical data for the prevalence of oxytetracycline resistance was generally the highest, and thus the model outputs derived from varying the environmental transmission over a range of plausible values were compared to the empirical prevalence of oxytetracycline resistance (Benedict et al., 2015; Noyes et al., 2015).

The model-estimated prevalence of oxytetracycline-resistant *M. haemolytica* was compared to empirical values to calibrate the respiratory transmission parameters, such as the hazard rate that calves would transmit the resistant organism. Each calibration was run for a minimum of 10,000 iterations over one year for *M. haemolytica*, on a 6,960-head feedlot. As described above, each iteration was run for minimum of six and maximum of 10 replications, and would cease if the estimated objective value fell within 95% confidence level around the mean of replication results (i.e., objective values) was met after the minimum number of replications, or following the maximum number of replications.

In a separate calibration experiment, a similar approach was used to calibrate two parameters – the coefficients of acquiring resistant *E. coli* and degradation of resistance reservoir. Each calibration was run for a minimum of 2,000 iterations over a period of three years on a 6,960-head feedlot). Similarly, each iteration was run for minimum of six and maximum of 10 replications (realizations). The replications would cease if the estimated objective value fell within 95% confidence level around the mean of replication results (i.e., objective values) was met after the minimum number of replications or following the maximum number of replications if the confidence level was not met. A warm-up period of more than one year was used to allow for the accumulation of AMR in the environment and carry over of AMR from one year to the next. Only the data from the end of the third year was used in the calibration experiment to match to the observed data. The total number of pens, iterations, and years were limited by the time necessary to run multiple year repetitions of this version of the model and the increasing complexity introduced by the environmental reservoir agent.

The final phase simultaneously estimated values accounting for both AMR selection due to drug use and AMR transmission due to fecal contamination with environmental transmission (fresh and residual feces containing AMR bacteria) and direct transmission of organisms among in contact calves (direct contact with contaminated hides or AMR feces). The AnyLogic® calibration experiments were repeated to estimate all relevant rates and transmission parameters for both oxytetracycline-resistant *M. haemolytica* and then again for *E. coli* with same number of iterations and replications for both the *E. coli* and *M. haemolytica* experiments as reported for previous steps.

After calibration was complete and using the calibrated parameters, a series of Monte Carlo experiments were conducted to obtain simulation outputs for the expected prevalence of tetracycline resistance at specific timepoints through the feeding period (1, 45, 90, 135, 180, 225 DOF) to compare the outputs resulting from each model configuration. For *M. haemolytica*, each experimental scenario was run for 2,000 iterations over a period of one year with 5 replications for each iteration to allow for stochastics and variability among specific sets of parameter values selected at the start of each iteration or model run. For example, the specific combination of drugs used for treatment varied with each iteration. With *E. coli*, 1,000 iterations were completed and reported over a period of three years due to time constraints.

3.3.5 Statistical analysis

Data processing and descriptive statistical analyses were performed using Microsoft Excel®. These included boxplots representing resistance prevalence over time, 2.5th and 97.5th percentiles. The range of data included in the scenario analysis was defined using the first quartile minus the 1.5 × the interquartile range as a lower bound, and the third quartile plus 1.5 × the interquartile range as an upper bound. Values observed outside of this range were classified as outliers. Analysis of observed oxytetracycline resistance was completed to estimate lower and upper 95% confidence intervals using the exact Clopper Pearson confidence interval method (Sergeant, 2018).

3.4 Results

Objective function values for *M. haemolytica* from drug use alone and the combination scenario were the same, while transmission alone had the lowest (best) objective function of three scenarios. For *E. coli*, drug use alone also had the largest (worst) objective function value, followed by the combination of drug use and transmission, with transmission alone being the lowest and best (Table 3.4).

Table 3.4: Objective function* values for both *E. coli* and *M. haemolytica* based on drug use alone, environmental transmission alone, or combination of both.

	Drug use only	**Transmission only**	**Drug use and transmission**
E. coli	0.1972	0.1308	0.1310
M. haemolytica	0.0139	0.0123	0.0139

***** The least-squared difference between model resistance prevalence output and observed resistance prevalence data collected from literature (Benedict et al., 2015; Noyes et al., 2015)

Mannheimia haemolytica

Each selection scenario followed a similar pattern for resistance prevalence and results were generally consistent with observed values collected from literature (Noyes et al., 2015). Resistance due to drug use alone or due to the combination of drug use and transmission increased from day 1 and peaked on day 90, before declining gradually over the rest of the feeding period. However, the peak of resistance prevalence due to transmission alone occurred on day 135 (Figure 3.2).

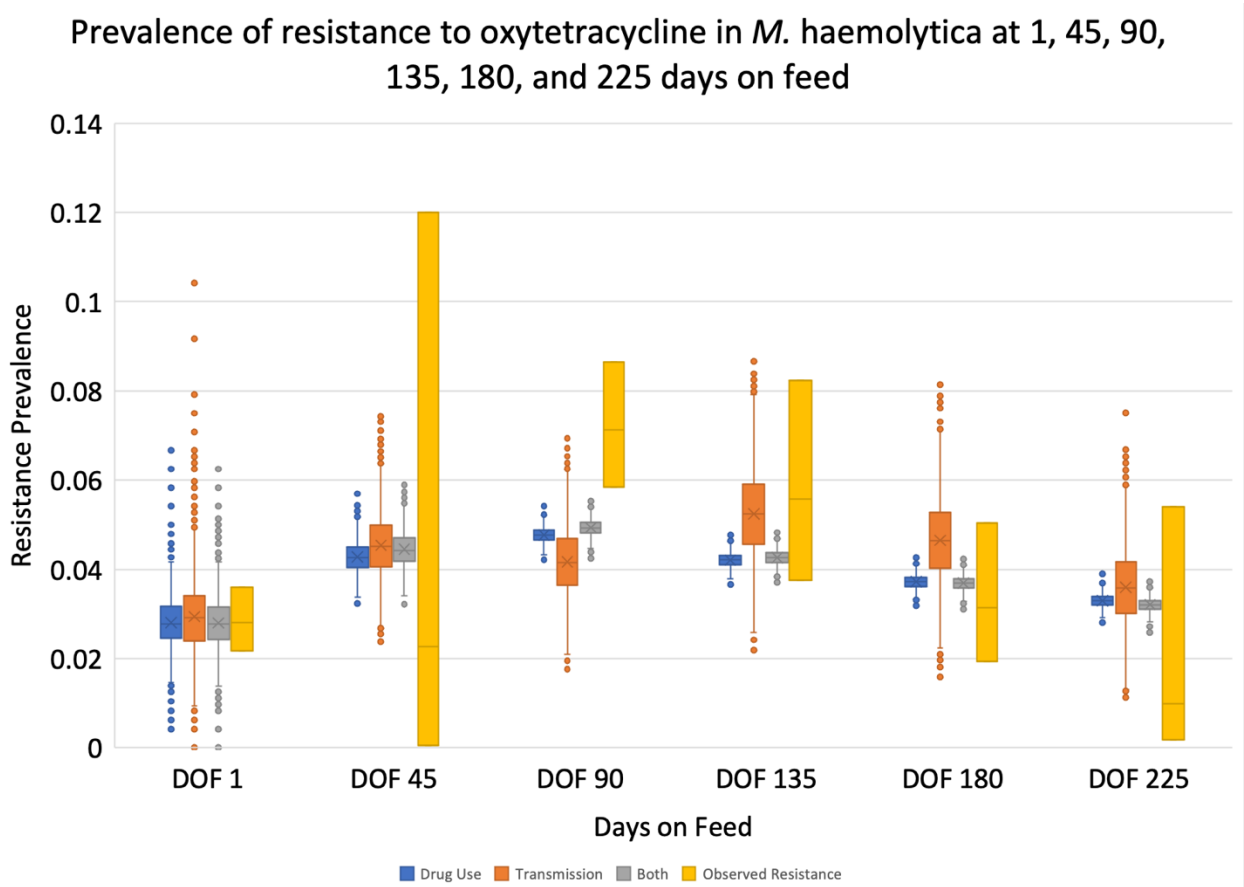

Figure 3.2: Tetracycline resistance prevalence in *M. haemolytica* due to drug use alone, environmental transmission alone, or combination of both. Line in each box plot represents the mean, the box boundaries represent the 25th and 75th percentiles, and the error bars reflect the lower and upper bounds defined based on 25th and 75th percentiles plus/minus 1.5 IQR. Individual outliers are represented by a single dot. Observed resistance plot boundaries reflect 95% confident intervals (Noyes et al., 2015; P. Morley personal communication, 2018).

Estimated values for tetracycline resistance prevalence for drug use only, transmission only and a combination of both overlapped with the 95% confidence limits for observed resistance values on all days with the exception of day 90 (Figure 3.2). On day 90, the highest resistance values for drug use only and a combination of both scenarios were less than the lowest value for observed resistance. However, day 90 resistance values for transmission only overlapped with observed resistance values.

Comparing 2.5th and 97.5th percentile ranges for the model outputs, all three scenarios overlap with the 95% confidence interval for observed prevalence values on days 1, 45, 180 and 225 (Table 3.5). On day 135, prevalence due to transmission only overlapped with observed prevalence values; prevalence due to drug use only or the combination of drug use and

transmission did not. Further, the 97.5th percentile for the observed prevalence values were lower than the lower bound of the 95th percent confidence interval for day 90 for all three scenarios.

In comparison to drug use only and a combination of both, selection due solely to transmission exhibited greater variability in resistance prevalence and the degree of dispersion was noticeably greater (Figure 3.2).

Table 3.5: Tetracycline resistance prevalence in *M. haemolytica* due to drug use alone, environmental transmission alone, or combination of both at 1, 45, 90, 135, 180 and 225 DOF.

DOF[†]	Drug use only		Transmission only		Drug use and transmission		Observed Resistance[*]
	2.5th percentile	97.5th percentile	2.5th percentile	97.5th percentile	2.5th percentile	97.5th percentile	(95% CI)
1	0.017	0.042	0.013	0.050	0.017	0.042	0.028 (0.021 – 0.036)
45	0.037	0.050	0.033	0.059	0.038	0.053	0.023 (0.0006 – 0.12)
90	0.044	0.051	0.027	0.057	0.046	0.053	0.071 (0.058 – 0.087)
135	0.039	0.045	0.034	0.071	0.039	0.046	0.056 (0.036 – 0.083)
180	0.034	0.040	0.029	0.065	0.034	0.040	0.031 (0.018 – 0.051)
225	0.030	0.036	0.020	0.053	0.029	0.035	0.010 (0.000 – 0.054)

*(Noyes et al., 2015; P. Morley, personal communication, 2018).

[†] Timepoints for 1, 45, 90, 135 180 and 225 DOF coincide with samples collected on arrival, and between 33 and 45, 46 and 90, 91 and 135, 136 and 180, and 181 and 225 DOF, respectively.

Escherichia coli

Tetracycline resistance values attributed to drug use alone were lowest on day 1 before sharply increasing to day 45. Following day 45, resistance plateaued and remained consistent until the end of the feeding period. Transmission and a combination of drug use and transmission were also lowest on day 1 before suddenly increasing to day 45. However, resistance in these scenarios then continued to increase gradually until the end of the feeding period, peaking on day 225 (Figure 3.3).

Tetracycline resistance values due to drug use alone overlapped with observed resistance values on day 1 (Figure 3.3) (Benedict et al., 2015). On day 45, tetracycline resistance prevalence for the drug use only, transmission only and a combination of both scenarios overlapped with the 95% confidence limits for observed resistance values on day 45. On days 90 to 225, resistance from transmission alone and the combination scenario overlapped with observed prevalence values, although values for drug use alone were consistently below the range for observed values (Figure 3.3).

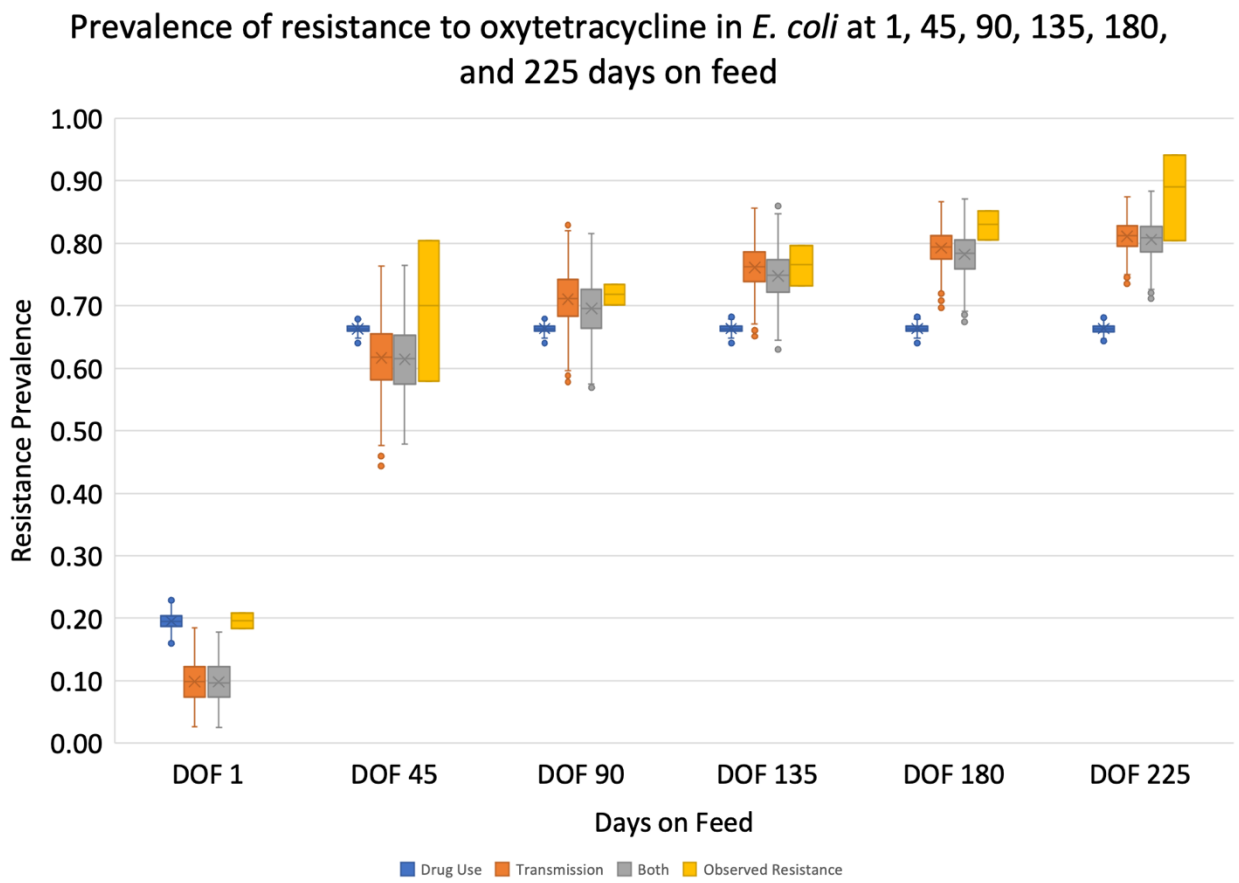

Figure 3.3: Tetracycline resistance prevalence in *E. coli* due to drug use alone, environmental transmission alone, or combination of both. Line in each box plot represents the mean, the box boundaries represent the 25th and 75th percentiles, and the error bars reflect the lower and upper bounds defined based on 25th and 75th percentiles plus/minus 1.5 IQR. Individual outliers are represented by a single dot. Observed resistance plot boundaries reflect 95% confident intervals calculated from the reported data (Benedict et al., 2015; P. Morley, personal communication, 2018).

The 95% CI for observed resistances overlapped with the estimated values (2.5th to 97.5th percentiles) for transmission alone and transmission and drug use from day 45 through 225, while the drug use alone had no overlap (Table 3.6).

Scenarios that included transmission expressed more variability in resistance prevalence compared to drug use alone, as indicated by the higher number of outliers and more extreme minimum and maximum values.

Table 3.6: Tetracycline resistance prevalence in *E. coli* due to drug use alone, environmental transmission alone, or combination of both at 1, 45, 90, 135, 180 and 225 DOF.

DOF[†]	Drug use only		Transmission only		Drug use and transmission		Observed Resistance[*]
	2.5th percentile	97.5th percentile	2.5th percentile	97.5th percentile	2.5th percentile	97.5th percentile	(95% CI)
1	0.172	0.219	0.038	0.159	0.038	0.163	0.196 (0.184 – 0.208)
45	0.651	0.675	0.509	0.718	0.509	0.713	0.700 (0.579 – 0.804)
90	0.652	0.675	0.624	0.792	0.604	0.781	0.718 (0.701 – 0.735)
135	0.652	0.675	0.690	0.828	0.670	0.821	0.766 (0.731 – 0.798)
180	0.652	0.675	0.733	0.845	0.712	0.845	0.830 (0.805 – 0.853)
225	0.651	0.676	0.759	0.854	0.746	0.862	0.890 (0.802 – 0.949)

*(Benedict et al., 2015; P. Morley, personal communication, 2018).

[†] Timepoints for 1, 45, 90, 135 180 and 225 DOF coincide with samples collected on arrival, and between 33 and 45, 46 and 90, 91 and 135, 136 and 180, and 181 and 225 DOF, respectively.

3.5 Discussion

The main question addressed in this analysis was a comparison of drug use to transmission (i.e., animal-to-animal contact, environmental contamination) in predicting oxytetracycline resistance in feedlot cattle. Direct transmission of AMR refers to transmission through contact between animals in close proximity, whereas indirect transmission involves an environmental component such as manure. In the current model, direct contact was assumed to be the primary route of *M. haemolytica* resistance transmission among animals. Further, resistant *E. coli* was assumed to accumulate in the environmental reservoir over time and could be transmitted via contact with manure. The model constructed herein was able to reasonably replicate observed historical oxytetracycline resistance for all three scenarios. Further, this is the first study to our knowledge to employ agent-based modelling to investigate the impact of AMU and contagious spread of AMR in feedlot cattle. Through this example, we were able to illustrate the utility of this tool for addressing the relationship between AMU and its relevance to AMR in feedlot cattle.

The model was not only able to mimic preventative and therapeutic treatment protocols as seen in typical western Canadian feedlots, but also allowed for stochasticity by incorporating disease incidence and contact between animals. Furthermore, the model was reasonably successful in comparing the role of drug use, transmission, or a combination of both on AMR in a feedlot setting. Prevalence of resistant *E. coli* from drug use only scenario was outside the observed real-world data from the literature except for on-arrival. For the transmission only and combination of drug use and transmission scenarios, *E. coli* resistance overlapped with observed resistance prevalence from the literature on days 90, 135, 180, and 225. Given these four timepoints are the most adjacent to harvest, the more limited fit earlier in the feeding period is relatively less important. For *M. haemolytica*, apart from day 90, the values from each scenario overlapped with observed real-world data from the literature. These results support that it is necessary to consider both drug use and transmission as they each represent an important determinant of AMR. The better fit for *M. haemolytica* model as compared with *E. coli* in this study most likely reflects the substantially lower number of iterations allowed for the *E. coli* models due to the need to run the models for three years and constraints on available run time and computational resources to complete this project.

Selection for resistant organisms associated with their competitive advantage with AMU has been recognized as an important determinant of AMR in feedlot cattle (Inglis et la., 2006; Checkley et al., 2010; Holman et al., 2018). However, previous work by Timsit et al. (2013) has demonstrated that contagious spread of organisms through physical contact can also occur. The authors illustrated that bulls from different feedlot pens were capable of acquiring identical *M. haemolytica* isolates following a single contact with infected calves at arrival. While the transmission can occur in a relatively short period of time, spread of contagious *M. haemolytica* could also depend upon management decisions made prior to feedlot induction such as frequency of contact.

There is also evidence that the natural environment serves as a possible reservoir and dispersal route of AMR pathogens or genes in humans (Huijbers et al., 2015; Wuijts et al., 2017). Similarly, livestock production contributes to the emergence and spread of antimicrobial resistance genes (ARGs) and antimicrobial residues in the environment through fecal excretion, resulting in the establishment of important AMR reservoirs (Singer et al., 2016; Thanner et al., 2016; Zaheer et al., 2019). This is supported by Mir et al. (2018) who reported a high prevalence of cefotaxime resistance in beef cattle raised in a facility without the use of injectable cephalosporins. This is also true with in-feed AMU. Müller et al. (2018) found that the effect of continued exposure to an environment in which macrolides had been administered in feed for extended periods of time played a bigger role than the use of injectable antibiotics. Finally, fecal-oral route has been shown to be an important mode of transmission. Stevenson et al. (2003) inoculated a subset of steers in three different pens with a rifampin-resistant *E. coli* strain, and within 48 hours of the introduction, the inoculated steers and penmates shed feces containing the resistant bacteria. This suggests that feces on the pen floor were the major source of resistant microorganism for re-infection of penmates. The current model is flexible and could be modified as necessary to account for scenarios involving specific conditions and interventions, such as changes in contact patterns, carry over reservoir and transmission likelihood.

A somewhat unexpected observation from the current model was that the objective function associated with drug use and transmission was not the lowest value. In other words, the combination of drug use and transmission produced a poorer fit of the model results to the observed prevalence data compared to transmission only. Granted, efficiently calibrating ABMs to real data is a substantial challenge. A large number of parameters are typically considered in

ABMs to capture the interactions and structure of complex systems. As a result, significant hardware requirements and computational costs are associated with searching for meaningful parameter combinations. Exploring the behaviour of the model through all possible parameter combinations while accounting for stochastic variability is very challenging (Lee et al., 2015). The current study maintained the when assessing resistance prevalence in each scenario for *M. haemolytica* and *E. coli*, despite each scenario varying in the number of parameters being calibrated. Therefore, it is possible that the objective function associated with the combination of drug use and transmission could have been lower if the number of iterations were increased accordingly.

Another important observation from the model is the difference in dispersion among the three scenarios. Specifically, scenarios involving transmission produced much greater variability in resistance prevalence compared drug use only. This is most likely due to the nature of transmission and the stochasticity associated with the probability of contact and the likelihood of acquiring resistance. Conversely, selection due to drug use only was dependent on a single rate resulting in less variability of data dispersion.

The current model made several simplifying assumptions that should be explored in future research. All calves within a single pen were assumed to arrive on the same day and only high-risk fall-placed calves were considered in this virtual feedlot. Bovine respiratory disease is a complex and multifactorial disease of cattle caused by stress, viruses and bacteria. *M. haemolytica* is the bacterium most frequently isolated from the lungs of cattle with BRD (Rice et al., 2007) and to simplify the model, *M. haemolytica* was assumed to be the primary bacterial pathogen causing BRD. Although BRD treatment failure can be attributed to several different factors (e.g., wrong drug-pathogen combination), it was assumed that failure was strongly impacted by the proportion of resistant *M. haemolytica* recovered at the pen level (Booker and Lubbers, 2020). At the time of model design and development, treatment failure rates for diseases such as arthritis and foot rot were not available. Therefore, therapeutic treatment was assumed to be effective, and the animal returned to a healthy state after the duration of therapy had lapsed. However, animals can be re-diagnosed with arthritis or foot rot following recovery.

The extent of animal-to-animal contact within the current model was also limited due to computational power and time requirements. An individual within a single pen was not able to transmit AMR to all other penmates in a single day and achieve adequate model speed. Instead,

each animal was assumed to transmit resistant bacteria to two random penmates every hour, for a total of 48 transmissions per day. In this scenario, each animal could potentially transmit to each one of its penmates over a period of five days. Further, transmission between pens was not considered and therefore results were limited to transmission between animals within the same pen.

Another limitation of the model was the reservoir accumulation. In a real-world setting, reservoir carryover between production cycles would exist and contribute to the overall environmental pool. The current model did not assume a baseline environmental reservoir at the beginning of the three-year cycle. This does not accurately reflect actual conditions as a feedlot pen is cleaned of manure after each production cycle, but never sterilized. However, the current model settings assumed an arbitrary 20% reservoir carryover following each production cycle to account for this limitation to some extent. Running the model for longer than three years might more closely reflect real world conditions.

Finally, data on feedlot cattle resistance prevalence utilized for this study was somewhat dated, with the most recent longitudinal studies reported by Noyes et al. (2015) and Benedict et al. (2015) coming from 2007. The Canadian Integrated Program for Antimicrobial Resistance Surveillance (CIPARS) has limited data on resistant *E. coli* in feedlot cattle for selected groups of antimicrobial agents (Government of Canada, 2020). However, longitudinal studies similar to the research conducted by Noyes et al. (2015) and Benedict et al. (2015) have not been repeated.

3.6 Conclusion

To summarize, an ABM describing AMR in feedlot cattle has been developed and the model showed that both AMU and contagious spread potentially contributed to AMR in feedlot cattle. This model provides a platform that can be refined as we gather additional data and can be used as a tool to identify knowledge gaps. Confined animal populations are unavoidably exposed to their manure and can become contaminated through contact with their surroundings in addition to direct contact with other animals. It is argued that without inclusion or consideration of all the pathways of AMR, mitigation plans are incomplete and may not improve existing and future conditions.

CHAPTER 4
GENERAL DISCUSSION AND CONCLUSIONS

Antimicrobial resistance poses a serious threat to disease control in both human and animal health with significant economic implications. The administration of antimicrobial agents in feedlot cattle raises a potential risk for the selection and spread of AMR. However, evidence illustrating the benefits of reduced AMU in feedlot cattle on the prevalence of resistant microorganisms is limited. Metaphylaxis is often criticized for providing the basis of selection of AMR in feedlot cattle, yet the effects of decreased metaphylactic AMU in feedlot cattle have not been systemically explored. It remains unclear whether reduced exposure to antimicrobial agents will result in a substantial reduction in AMR. Early disease management may reduce the overall number of sick animals while decreasing the amount of antimicrobials required for therapy later in the feeding period. This has been shown to increase profitability and improve animal welfare, but the impacts on AMR require further study.

Chapter 2 sought to model the influence of tulathromycin and oxytetracycline metaphylactic AMU compared to no metaphylaxis on AMR in feedlot cattle. The results illustrated a significantly lower resistance prevalence to phenicols and trimethoprim sulfadoxine (TMS) when tulathromycin and oxytetracycline were administered as metaphylaxis compared to no metaphylaxis. The biggest observed magnitude of differences were with respect to resistance to TMS, the choice for treatment after two previous BRD treatment failures. While lower resistance prevalence associated with metaphylaxis was observed with antimicrobials from different drug classes than those used to treat BRD, metaphylactic AMU did increase resistance for antimicrobials within the same drug class. Oxytetracycline resistance was expected to increase due to selective pressure when used as on-arrival metaphylaxis. However, no drugs from the tetracycline class were administered as therapeutic treatment for BRD in present model, and so reductions to the incidence of BRD would not equate to decreased therapeutic oxytetracycline use. In-feed chlortetracycline use provides further opportunity for resistance selection of tetracycline, and therefore tetracycline had the greatest resistance prevalence for the oxytetracycline metaphylaxis, rather than the control group as observed in the other drug classes.

Metaphylactic AMU has been shown to reduce BRD cases in feedlot cattle (Ives and Richeson, 2015; Abell et al., 2017; Lhermie et al., 2019). It is plausible that the reduction to

resistance prevalence was due to fewer incidences of illness requiring therapeutic AMU for BRD. These downstream effects have important implications in relation to AMR in feedlot cattle. There are differences among drug classes used in the model in terms of their relative importance to human health. Specific drug classes, such as TMS, are considered more important than others, such as florfenicol and tetracycline, in the treatment of serious human medicine bacterial infections. Any opportunity for resistance selection of those antimicrobials from the beef industry could have public health consequences. In the current study, the use of metaphylaxis resulted in decreased TMS resistance in *E. coli* and *M. haemolytica*. This suggests that fewer antimicrobials from this class of higher relative importance to human health were used due to fewer treatment failures.

Together, these observations imply a potential feedlot management approach to reduce opportunities for resistance selection pressures of antimicrobials. The absence of metaphylactic AMU in high-risk calves arriving at a feedlot could result in greater disease incidence and subsequently higher use of MIAs for therapeutic treatment. Therefore, current result suggested that metaphylaxis is warranted to reduce disease incidence throughout the feeding period. Since an increase in resistance prevalence was observed within the same drug class as the antimicrobial administered for metaphylaxis, products that have lower importance to human medicine (e.g., tetracyclines) should be selected to minimize the potential public health impact.

The model also considered the influence of AMU to treat arthritis and foot rot on AMR. Lameness is a major cause of disease and production losses for feedlot cattle (Marti et al., 2016; Davis-Unger et al., 2017, 2019), resulting in increased days required to finish calves, greater drug expenses, additional labour for treatments and higher mortality rates (Terrell et al. 2014). In the current model, tulathromycin and oxytetracycline were both drug treatment options for arthritis. Therefore, contributions from AMU on AMR were expected, particularly when the same drug classes were used as metaphylaxis. However, therapeutic treatment for lameness was assumed to be 100% effective, whereas there would be treatment failure expected in a real-world setting. While this was a simplification, the effect of AMU to treat arthritis and foot rot on overall AMR was adequately represented for the purpose of modeling AMR dynamics in feedlot cattle. BRD (cumulative incidence, 6.4%) has been reported to account for substantially more AMU in a large study of high and low risk calves than either foot rot (cumulative incidence,

3.5%) or infectious arthritis (cumulative incidence, 0.8%) and, as such, was the focus of this investigation and warranted more precision in modeling (Davis-Unger et al., 2019).

Multi-drug resistance remains a challenging contributing to AMR, despite the use of antimicrobials with low importance to human medicine. Even with the administration of antimicrobials of low importance, the co-selection potential for MDR microorganisms containing resistance to MIAs exists (Sundqvist et al., 2010; Klima et al., 2014b). However, this was not considered in the current model as the current proof of concept analysis already contained a substantial degree of complexity.

Multidrug resistance has been recently reported in *M. haemolytica* isolates obtained from cattle (Lubbers and Hanzlicek, 2013; DeDonder and Apley, 2015; Woolums et al., 2018). Many of the resistance genes in these isolates are associated with ICEs which can harbor extensive MDR cassettes (Klima et al., 2014b, 2020; Eidam et al., 2015; Cameron et al., 2019; Stanford et al., 2020). While MDR has not historically been observed as an issue in feedlot cattle, strains of *M. haemolytica* resistant to more than one class of antimicrobials have been reported in recent studies (Lubbers and Hanzlicek, 2013; Anholt et al., 2017; Snyder et al., 2017, 2019; Timsit et al., 2017; Woolums et al., 2018). Further, *M. haemolytica* shed by high-risk calves that have been previously treated is associated with an elevated potential for MDR. This represents an important connection of MDR to metaphylactic AMU strategies used in feedlot cattle.

Although fewer studies evaluating MDR in *E. coli* have been conducted, recent research has indicated that over 98 and 93% of isolates from feedlot calves were resistant to fewer than or equal to three antimicrobials when sampled on arrival and at hormone implantation, respectively (Benedict et al., 2015). Combined with observations of resistant *M. haemolytica* described above, this represents a potential public health risk as a source for spread of MDR determinants from feedlots (Geser et al., 2012; Szmolka and Nagy, 2013). Therefore, the omission of MDR in the current model limits our understanding of resistance transmission and the best strategies for antimicrobial stewardship. Due to the computational limitations and lack of corresponding data from feedlots, MDR was not considered in the current model. However, MDR is a potential important contributing factor where the use of a non-MIA products can still result in selection of MIA. The possibility of co-selection of AMR may have far-reaching implications and suggests that further research should be undertaken to investigate.

There are other important factors influencing AMR beyond AMU alone. In Chapter 2, the model investigated the influence of metaphylactic AMU on AMR in feedlot cattle. However, environmental transmission from either a reservoir of resistant bacteria, or via animal-to-animal contact, also represents a significant factor for AMR. Therefore, Chapter 3 attempted to address the role of the environment on AMR in feedlot cattle.

A reservoir of resistance can also be transmitted indirectly via environment and direct contact among animals, and the model successfully demonstrated their influence on AMR in a feedlot setting. Understanding the role that the environment plays in the dissemination of AMR is necessary to identify any modifiable aspects to reduce or interrupt the spread of AMR. Previous studies have indicated that the natural environment serves as a possible reservoir and dispersal route of AMR pathogens or genes in human (Huijbers et al., 2015; Wuijts et al., 2017). With regular AMU contributing to the emergence and spread of ARGs, feedlots serve as important AMR reservoirs (Singer et al., 2016; Thanner et al., 2016; Zaheer et al., 2019). In-feed AMU (Müller et al., 2018) and the fecal-oral route (Stevenson et al., 2003) also contribute to the accumulation of an AMR reservoir and subsequent transmission of resistance. Chapter 3 results implied that transmission alone produced a better fit of the model results to observed data compared to both drug use and transmission. We had expected to produce a better model fit when combining drug use and transmission, although other model limitations could explain this discrepancy.

Efficiently calibrating ABMs to real data is difficult to achieve given the large number of parameters required order to capture the interactions and structures of complex systems. This results in significant hardware requirements and high computational costs, making it practically impossible to consider all potential parameter combinations (Lee et al., 2015). The same number of calibration iterations were used for drug use only, transmission only or a combination of both when assessing resistance prevalence, although the number of parameters being calibrated for each varied. Therefore, it is possible that the objective function associated with the combination of drug use and transmission may have been lower if more iterations were performed. Furthermore, it is challenging to parameterize an ABM because the data available from field studies are not necessarily directly correlated with the types of interactions that are being modeled. This is especially true of AMU, where a wide variety of antimicrobial agents are available as treatment options.

Another model limitation relating to environmental transmission was the accumulation of the resistance reservoir. Reservoir carryover between feedlot production cycles would exist in reality and contribute to the overall environmental pool. Due to an absence of data from the literature describing how reservoirs accumulate over time, a baseline environmental reservoir at the beginning of the three-year cycle was not implemented. In a real-world setting, feedlot pens are cleaned of manure after each production cycle, but never sterilized, and thus this parameter was simplified in the current model. The current model settings arbitrary assigned a 20% reservoir carryover following each production cycle as sterility did not exist in the feedlot environment. The idea of starting from a new feedlot is consistent with another AMR study currently being completed at the new University of Saskatchewan feedlot. That feedlot is just starting its third cycle and it is hoped that data from the project can further inform this model in the future.

Animal-to-animal contact modeled in the current study was also limited due to computational power and time requirements. Individual animals were assumed to transmit resistant bacteria to two random penmates every hour, for a total of 48 transmissions per day. In this scenario, each animal could potentially transmit to each one of its penmates over a period of five days. However, it is more likely that animals could contact each of their penmates over a single 24-hour period in a real-world setting. Further, transmission between pens was not considered and therefore results were limited to transmission between animals within the same pen. Despite this limitation, the simplified transmission dynamics were adequate for modeling the influence of animal-to-animal environmental AMR transmission.

The ABM developed here can be adapted to address several of these limitations should applicable data and literature become available. As with the current study, future ABM applications to livestock research can also help to identify knowledge gaps, enabling empirical investigators to focus their efforts on identifying modifiable interactions most likely to aid our understanding of AMR. The present study illustrated that metaphylactic AMU restrictions may not be an uncomplicated solution for reducing AMR, while metaphylaxis remains an effective way to reduce morbidity and mortality related to BRD due to the potential for an overall reduction in MIA used for BRD therapy. While macrolides are considered an MIA and are the most common class of drug used for metaphylaxis in high-risk cattle, options to more frequently consider oxytetracycline use when supported by evidence could be a feasible option recognizing

the relative benefits of most macrolides over tetracyclines in managing BRD (O'Connor et al. 2019).

The stress of transition for young calves into feedlots could also represent a substantial influence on AMR (Doster et al., 2018). Therefore, management practices that better prepare animals for feedlots should be sought and implemented in addition to metaphylactic efforts. Preconditioning is a collection of management practice that promote greater resilience in calves to the stressors associated with arriving at the feedlot (Richeson and Falkner, 2020). Vaccination, low-stress weaning, performing surgical procedures (such as dehorning, castration) as neonates, and bunk training are some of the management practices that aim to reduce the stress of cattle prior to their arrival at the feedlot (Taylor et al., 2020), although there are no current economic incentives for producers who adopt these practices. Another approach that would promote reduced AMR transfer involves increased cleaning of common areas, such handling facilities and chute systems. This management practice would reduce the reservoir of resistant and disease-causing bacteria available for transfer to subsequent animals handling. While metaphylactic AMU remains the most effective approach for controlling disease in new-arrival feedlot cattle, utilizing antimicrobials with lower importance to human medicine could potentially reduce the risk of AMR to MIAs.

An ABM was successfully constructed to model AMR dynamics in a western Canadian feedlot setting. Antimicrobial resistance is often characterized by stochastic, non-linear, and dynamic interactions between individuals and the environment. These complex features cannot be adequately captured with traditional statistical models or models based on sets of ordinary differential equations. Agent-based modeling has the ability to overcome some limitations imposed by conventional modelling approaches, such as lack of stochasticity and homogeneity. However, the application of ABMs to AMR in agriculture and food system settings is still very limited (Ramsay et al., 2018). To our knowledge, the current study is the first to have ever utilized ABM to model AMR in a virtual beef feedlot setting. Agent-based models are particularly well suited to study AMR since the behaviour of the system arises partly from individual-level behaviour and interactions among individuals. Furthermore, AMR involves interactions through time, where past actions affect the future decision-making context in a feedback loop. In summary, ABMs provide a means for generating a theory, testing hypotheses,

and extending traditional experimental approaches by facilitating the investigation of complex problems such as AMR epidemiology in feedlot cattle.

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